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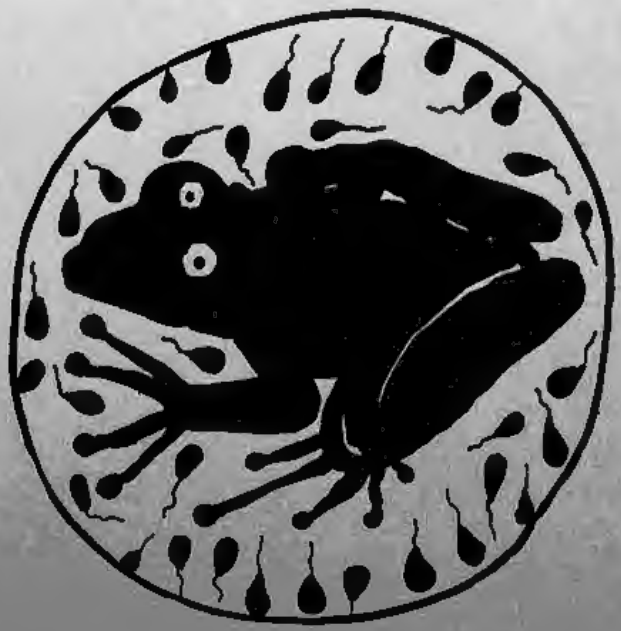

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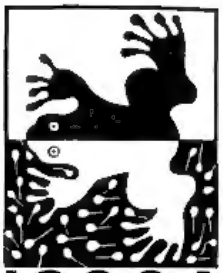

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Comparative morphology of phytotelmonous and pond-dwelling larvae of four neotropical treefrog species (Anura, Hylidae, *Osteocephalus oophagus*, *Osteocephalus taurinus*, *Phrynohyas resinifictrix*, *Phrynohyas venulosa*)

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We dedicate this paper to the memory of Rudolf WYTEK († August 25, 1995, Vienna)

External and buccopharyngeal morphology of phytotelmonous and pond-dwelling larvae of four neotropical hylid species were analyzed with respect to differential diagnosis and ecomorphology. Larvae typically live in bromeliads (*Osteocephalus oophagus*), tree holes (*Phrynohyas resinifictrix*), ponds in rainforest (*Osteocephalus taurinus*), and ponds in open areas (*Phrynohyas venulosa*). Discriminant analysis of morphometric external characters revealed only slight differences between the phytotelmonous species, but two well separated subgroups among the pond-dwelling species. All species showed average body proportions, macrophagous habits, and pulmonary as well as branchial respiration. Tadpoles living in phytotelms were characterized by reduction of peribuccal and buccopharyngeal structures, and differed notably in jaw sheath morphology and lung development among species. Tadpoles developing in ponds were characterized by high numbers of tooth rows and low balance values. The genera *Osteocephalus* and *Phrynohyas* were distinguished best by development of secondary upper tooth rows, position of the eyes, and gross body proportions.

INTRODUCTION

In this study we describe the tadpoles of *Osteocephalus oophagus* Jungfer & Schiesari, 1995, *Osteocephalus taurinus* Steindachner, 1862, *Phrynohyas resinifictrix* (Goeldi, 1907), and *Phrynohyas venulosa* (Laurenti, 1768). Larval gross body characters and external buccal features are examined through series comprising almost the entire larval period. Internal buccopharyngeal surface features and detailed morphometry are considered in advanced tadpole stages only.

Tadpoles of the four species have already been described by a number of authors (Table I). However, with the exception of *P. venulosa*, those descriptions were based on small samples and provided little information on ontogenetic and intraspecific variation. Information on buccopharyngeal larval morphology was available only for *P. resinifictrix*.

The neotropical hylid genera *Osteocephalus* Steindachner, 1862 (seven species; JUNGFER & SCHIESARI, 1995) and *Phrynohyas* Fitzinger, 1843 (five species; FROST, 1985) are well defined by their adult morphology (TRUEB, 1970; TRUEB & DUELLMAN, 1971). Within both genera, larval habitats vary significantly among species and include phytotelms, ponds, and streams (Table VIII). The larval habitats for the four species in this study are principally of two types: phytotelms and ponds. Larvae of *O. oophagus* typically develop in rainforest bromeliads at the ground or off-ground up to 2 m high; those of *P. resinifictrix* dwell in spacious tree holes in the canopy up to 35 m high. In contrast, larvae of *O. taurinus* typically develop in rainforest ponds and those of *P. venulosa* in ponds of open areas.

To better understand larval morphology of the species analyzed in depth in this study, we finally include comparative data from the literature on other *Osteocephalus* and *Phrynohyas* species (Table VIII, fig. 7) and refer to phenotype-ecotype interdependencies recognized for anuran larvae (WASSERSUG, 1980; LANNOO et al., 1987; WASSERSUG & HEYER, 1988; ALTIG & JOHNSTON, 1989).

MATERIAL AND METHODS

Collection data, as well as the number and range of developmental stages of the tadpoles examined, are summarized in Table II. Tadpoles were preserved in 4 % formaldehyde solution. Most of the series were preserved in the field shortly after collection. Some series of *P. resinifictrix* and *O. taurinus* were reared in the laboratory from spawn or tadpoles collected in the field. Specific assignment of the tadpoles collected by L. C. SCHIESARI was confirmed by specimens raised in the laboratory either from identified spawn or until neometamorphosed stages (exceeding stage 46, GOSNER, 1960), and was confirmed by congruency with the museum tadpole series indicated in Table II. *P. venulosa* larvae were identified by J. P. CALDWELL.

Determination of larval developmental stages follows GOSNER (1960). Morphometric parameters (Table III) are defined as in GRILLITSCH et al. (1993). Within stages, the

Table I. - Literature survey on larval morphology of *Osteocephalus oophagus*, *Osteocephalus taurinus*, *Phrynohyas resinifictrix* and *Phrynohyas venulosa*. Information present (+) or not present (-).

Species	Stages	Localities	Features							References
			General appearance		Oral disk		Buccopharyngeal cavity		Mor-phometry	
			Described	Illustrated	Described	Illustrated	Described	Illustrated		
O. oophagus	37	Manaus, Amazonas, Brasil	-	+	-	-	-	-	-	HERO (1990) [1]
	19-40	Manaus, Amazonas, Brasil	+	+	+	-	-	-	+	JUNGFER & SCHIESARI (1995)
O. taurinus [2]	40-41	Manaus, Amazonas, Brasil	-	+	-	+	-	-	-	HERO (1990)
P. resinifictrix		-	+	-	+	-	-	-	-	LANNOO et al. (1987)
	39	Manaus, Amazonas, Brasil	-	+	-	+	-	-	-	HERO (1990)
	28-38	Panguana, Perú	+	+	+	+	+	+	+	GRILLITSCH (1992)
P. venulosa	18-41	Bejuco, Panamá, Panamá	+	+	+	+	-	-	+	ZWEIFEL (1964)
	17-46	Encinal, Veracruz, México	+	+	+	+	-	-	+	PYBURN (1967) [3]
	38	Encinal, Veracruz, México	+	+	+	+	-	-	-	DUELLMAN (1970) [4]
	38	Leticia, Colombia	+	-	+	-	-	-	-	DUELLMAN (1978)
		Río Negro, Edo. Miranda, Venezuela	+	+	+	+	-	-	-	RADA DE MARTINEZ (1990)

1. Tadpoles described under the name *Osteocephalus* sp.
2. Description of *O. taurinus* tadpoles in DUELLMAN & LESCURE (1973) must be referred to *Hyla geographica* (CALDWELL, 1989); this also applies to the *O. taurinus* tadpole described in DUELLMAN (1978). Original description of *Hyla elkejungingerae* (HENLE, 1981) includes larval morphology. This species has been considered a possible synonym of *O. taurinus* (FROST, 1985), but was redefined by HENLE (1992) as *Osteocephalus elkejungingerae* and is considered as a distinct species in the present study.
3. Tadpoles described under the name *Phrynohyas spilomma*.
4. Reexamination of the series of ZWEIFEL (1964) and PYBURN (1967), and comparison with a further series from Tepic, Nayarit (México), support conspecificity of the tadpoles of ZWEIFEL and PYBURN.

Table II. - Material investigated: Museu Nacional do Rio de Janeiro, Brasil (MNRJ); Museu de Zoologia da Universidade de São Paulo, Brasil (MZUSP); L. C. SCHIESARI field numbers of material deposited in the MZUSP (LCS).

Localities: Reserva Florestal Adolfo Ducke, Manaus, Amazonas, Brasil (A); Reservas INPA No. 3402, 1501, 1401, Instituto Nacional de Pesquisa do Amazonas - World Wildlife Fund, Amazonas, Brasil (B); Juruá, Rio Xingú, Pará, Brasil (C); Boa Vista, Roraima, Brasil (D).

Collectors: see notes.

Morphometry: number of specimens and range of stages of the specimens investigated for gross body morphometry (Table III), detailed morphometry (Table III) and tooth row counts (Table IV).

Specimen series	Localities (collectors)	Dates of collections	Gross morphometry		Detailed morphometry		Tooth row counts	
			Numbers of specimens	Ranges of stages	Numbers of specimens	Ranges of stages	Numbers of specimens	Ranges of stages
O. oophagus	A [4]							
LCS 345		26.01.93	7	27-37	7	37-39	8	27-37
LCS 361 [1, 2, 3]		09.03.93	20	31-40	1	37	17	31-40
O. taurinus								
MNRJ 7971 [1, 2]	A [5]	26.10.85	6	35-39	3	37-39	6	35-39
MZUSP 66336	B [6]	01.-05.93	3	36-39	2	39	3	36-39
LCS 343 [3]	A [4]	27.01.93	33	25-26	-	-	34	25-26
LCS 364 [3]	A [4]	12.03.93	17	27-28	-	-	16	27-28
P. resinifictrix	A [4]							
LCS 342 [3]	Tree 1	21.01.-11.03.93	6	30-36	-	-	5	30-36
LCS 355	Tree 1	18.02.93	9	25-26	-	-	4	25-26
LCS 362	Tree 1	10.03.93	2	25	-		2	25
LCS 372-375 [1]	Tree 1	13.02-12.06.93	30	25-40	3	37	19	26-41
LCS 347	Tree 2	28.01.93	8	26-35	-	-	7	26-35
LCS 356	Tree 2	18.02.93	6	27-31	-	-	4	27-30
LCS 376-377 [2]	Tree 2	29.01.-17.04.91	17	25-39	3	37-39	16	25-39
LCS 378 [1]	Tree 3	29.01.91	14	25-37	1	37	12	26-37
LCS 366	Tree 4	18.03.93	2	25	-	-	2	25
LCS 379	Tree 5	30.01.91	5	25	-	-	4	25
LCS 357 [3]	Tree 6	24.02-09.03.93	8	27-37	2	37	8	27-37
P. venulosa	C [7]							
MZUSP 64359		15.01.87	5	30-34	-	-	5	20-34
MZUSP 64360 [1]		1987	2	35-37	1	37	2	35-37
MZUSP 64361 [1, 2]		27.01.87	4	37-40	3	37-38	-	-
MZUSP 64364		05.02.87	1	39	2	39	-	-
MZUSP 64367		14.02.87	2	25	-	-	3	25
MZUSP 68933	D [7]	23.06.91	2	36-37	1	37	2	36-37

1. Samples used for SEM analysis of buccopharyngeal cavity.
2. Samples used for SEM analysis of oral disk.
3. Samples raised in the laboratory.
4. Coll. L. C. SCHIESARI.
5. Coll. J.-M. HERO.
6. Coll. C. GASCON.
7. Coll. J. P. CALDWELL.

Table III. - Comparison of morphometric parameters describing the body proportions of the tadpoles of *Osteocephalus oophagus*, *Osteocephalus taurinus*, *Phrynohyas resinifictrix* and *Phrynohyas venulosa*, for developmental stages pooled within two different ranges.

Parameters (distances as defined in GRILLITSCH et al., 1993): maximum diameter of eye (ED); maximum height of tail (HT); maximum height of lower tail fin (LF); internarial distance (NN); naro-pupillar distance (NP); maximum width of oral disk (OD); interpupillar distance (PP); rostro-narial distance (RN); distance tip of snout - opening of spiracle (SS); distance tip of snout - insertion of upper tail fin (SU); distance snout - vent, snout-vent length (SV); maximum height of upper tail fin (UF); distance vent - opening of spiracle (VS); distance vent - tip of tail, tail length (VT).

For each parameter, the following information is provided: mean value ± standard deviation; range in parenthesis; number of specimens in brackets; coefficient of regression of ratios with total length / Spearman correlation coefficient of ratios with stage in waved brackets, * $P < 0.05$, ** $P < 0.01$.

Parameters		Stages pooled	*O. oophagus*	*O. taurinus*	*P. resinifictrix*	*P. venulosa*
Vent - tail tip / snout - vent	VT / SV	26 - 40	1.20 ± 0.13 (0.82-1.47) [27] {0.0000/-0.25}	1.36 ± 0.08 (1.22-1.52) [35] {0.0017/0.01}	1.47 ± 0.11 (1.25-1.72) [87] {-0.0030/0.12}	1.38 ± 0.08 (1.26-1.50) [14] {0.0061/0.41}
		36 - 40	1.17 ± 0.14 (0.82-1.41) [12]	1.38 ± 0.09 (1.22-1.49) [7]	1.47 ± 0.12 (1.25-1.70) [18]	1.41 ± 0.08 (1.26-1.50) [8]
Vent - tail tip / tail height	VT / HT	26 - 40	2.42 ± 0.26 (1.99-3.07) [23] {0.0216/0.05}	3.26 ± 0.29 (2.77-4.23) [30] {0.0075/-0.01}	2.53 ± 0.26 (2.06-3.05) [47] {0.0070/0.16}	2.50 ± 0.18 (2.14-2.75) [14] {-0.0111/-0.32}
		36 - 40	2.47 ± 0.31 (2.04-3.07) [10]	3.60 ± 0.34 (3.20-4.23) [5]	2.55 ± 0.12 (2.34-2.83) [12]	2.44 ± 0.18 (2.14-2.69) [8]
Tail height / upper fin height	HT / UF	26 - 40	3.15 ± 0.15 (2.90-3.41) [24] {0.0025/0.26}	3.44 ± 0.28 (3.05-4.21) [30] {0.0074/-0.31 *}	3.10 ± 0.29 (2.71-3.71) [50] {0.0329 **/ 0.15}	2.84 ± 0.11 (2.63-3.03) [16] {0.0023/-0.02}
		36 - 40	3.19 ± 0.14 (2.94-3.41) [10]	3.63 ± 0.39 (3.06-4.21) [5]	3.07 ± 0.25 (2.74-3.56) [13]	2.82 ± 0.10 (2.63-2.94) [9]
Upper fin height / lower fin height	UF / LF	26 - 40	1.07 ± 0.07 (0.92-1.19) [24] {0.0085 **/0.48 **}	1.03 ± 0.09 (0.79-1.16) [30] {0.0039 */0.18}	0.99 ± 0.09 (0.79-1.16) [50] {0.0023/0.35 **}	0.98 ± 0.06 (0.86-1.10) [16] {-0.0033/-0.16}
		36 - 40	1.10 ± 0.06 (0.99-1.19) [10]	1.11 ± 0.07 (0.99-1.22) [5]	1.02 ± 0.08 (0.90-1.16) [13]	0.97 ± 0.05 (0.86-1.04) [9]
Snout - vent / snout - upper fin	SV / SU	26 - 40	1.66 ± 0.15 (1.32-2.15) [27] {-0.0063/-0.17}	1.51 ± 0.08 (1.37-1.71) [31] {0.0000/0.11}	1.65 ± 0.20 (1.36-2.19) [50] {0.0062/0.22}	2.07 ± 0.20 (1.81-2.39) [15] {0.0079/0.22}
		36 - 40	1.65 ± 0.19 (1.32-2.15) [12]	1.50 ± 0.09 (1.44-1.66) [4]	1.72 ± 0.18 (1.42-1.93) [13]	2.16 ± 0.22 (1.84-2.39) [8]
Snout - spiracle / vent - spiracle	SS / VS	36 - 40	1.20 ± 0.15 (0.98-1.49) [8]	1.23 ± 0.17 (0.94-1.46) [5]	1.18 ± 0.15 (0.94-1.41) [9]	1.35 ± 0.16 (1.12-1.64) [7]
Interpupillar / internarial	PP / NN	36 - 40	1.51 ± 0.05 (1.43-1.59) [8]	1.42 ± 0.06 (1.36-1.51) [5]	1.67 ± 0.07 (1.51-1.75) [9]	1.51 ± 0.04 (1.45-1.58) [7]
Rostro-narial / naro-pupillar	RN / NP	36 - 40	1.03 ± 0.10 (0.85-1.20) [8]	0.92 ± 0.14 (0.73-1.15) [5]	0.78 ± 0.15 (0.53-1.13) [9]	0.86 ± 0.15 (0.63-1.04) [7]
Interpupillar / oral disk width	PP / OD	36 - 40	1.61 ± 0.06 (1.55-1.74) [8]	1.51 ± 0.09 (1.36-1.62) [5]	2.24 ± 0.12 (2.09-2.43) [9]	1.87 ± 0.06 (1.81-1.96) [6]
Internarial / oral disk width	NN / OD	36 - 40	1.07 ± 0.04 (1.03-1.15) [8]	1.06 ± 0.07 (0.99-1.16) [5]	1.34 ± 0.08 (1.22-1.45) [9]	1.25 ± 0.03 (1.21-1.28) [6]
Interpupillar / eye diameter	PP / ED	36 - 40	3.24 ± 0.26 (2.92-3.63) [8]	3.15 ± 0.22 (2.88-3.49) [5]	4.17 ± 0.42 (3.54-4.85) [9]	3.92 ± 0.07 (3.78-3.99) [7]

Table IV. - Comparison of collective median formulae (DUBOIS, 1995) of labial tooth rows (median values, range in parentheses, number of specimens in brackets, presence and position of gaps not indicated) of *Osteocephalus oophagus*, *Osteocephalus taurinus*, *Phrynohyas resinifictrix* and *Phrynohyas venulosa*, in relation to the stage of larval development.

Stages	Median collective tooth row formulae							
	O. oophagus		*O. taurinus*		*P. resinifictrix*		*P. venulosa*	
25		-	2 / 3 (3-4)	[27]	2 / 3 (3-4)	[15]	2 / 3	[3]
26		-	2 / 4 (3-4)	[10]	2 / 3;4 (3-4)	[16]		-
27	2 / 3	[1]	2 / 5	[5]	2 / 4 (3-4)	[11]		-
28	2 / 3	[1]	2 / 5 (5-6)	[11]	2 / 4	[5]		-
29	2 / 3	[1]		-	2 / 4 (3-4)	[3]		-
30	2 / 3	[1]		-	2 / 4 (3-5)	[6]	3 / 4	[1]
31	2 / 3	[3]		-	2 / 4 (3-4)	[6]	3 / 4	[3]
32		-		-	2 / 5	[1]		-
33	2 / 3	[2]		-	2 / 4	[2]		-
34	2 / 3	[2]		-	2 / 4 (3-4)	[3]	3 / 5	[1]
35	2 / 3	[3]	2 / 6	[1]	2 / 4	[2]	3 / 5	[1]
36	2 / 3	[1]	2 / 5 (4-6)	[3]	2 / 4	[4]	4 / 5	[1]
37	2 / 3	[2]	2 / 7;6 (6-7)	[2]	2 / 4	[8]	3 / 5 (4-5)	[4]
38	2 / 3	[3]		-		-	3 / 4	[1]
39	2 / 3	[4]	2 / 6 (5-6)	[3]	2 / 4 (3-4)	[3]	3 / 5	[2]
40	2 / 3	[1]		-	2 / 4	[4]	3 / 5	[1]
41		-		-	2 / 4	[1]		-

collective labial tooth row formula (DUBOIS, 1995) is described by median value and range of tooth row counts in the anterior and posterior labium, respectively. Balance values of tooth row counts (i.e., number of rows in the upper labium minus number of rows in the lower labium) are according to ALTIG & JOHNSTON (1989), who categorized tooth row formulae as balanced (equal number of rows on upper and lower labia), negatively imbalanced (more rows on lower labium), or positively imbalanced (more rows on upper labium). Terminology of jaw sheath morphology follows, e.g., KAUNG & KOLLROS (1976) and FOX (1984). Terminology of buccopharyngeal structures is in accordance with WASSERSUG (1976, 1980) and WASSERSUG & HEYER (1988). Typological assignment of breeding habitats (Table VIII) corresponds to DUELLMAN & TRUEB (1985), LANNOO et al. (1987), DUELLMAN (1988), and ALTIG & JOHNSTON (1989).

The tadpoles analyzed comprise a wide range of developmental stages (Table II). For all specimens, developmental stage, total length (fig. 1), gross body proportions (Table III), and tooth row counts (Table IV) were determined. Investigations on further morphometric parameters (Tables III and V) and on buccopharyngeal structures were

Table V. - Comparison of (A) external and (B) internal features of *Osteocephalus oophagus*, *Osteocephalus taurinus*, *Phrynohyas resinifictrix* and *Phrynohyas venulosa* in advanced larval developmental stages (38 ± 2, numbers of specimens according to those in Tables II and IV).

A. External features.

Parameters	*O. oophagus*	*O. taurinus*	*P. resinifictrix*	*P. venulosa*
Mean total length (mm)	29.7	32.7	37.0	41.8
Maximum total length (mm, present study)	36.2 (stage 40)	38.3 (stage 39)	47.0 (stage 39)	39.9 (stage 39)
Maximum total length (mm, from the literature) [1]	26.0 (stage 37)	31.7 (stage 40)	38.7 (stage 39)	49.4 (stage 41)
Labial tooth row formula (present study) [2]	2 / 3	2 / 6 (4-7)	2 / 4 (3-4)	3 (3-4) / 5 (4-5)
Labial tooth row formula (from the literature) [1, 2]	2 / 3	2 / 6 (4-7)	2 / 4 (3-4)	3 (3-4) / 5 (4-7)
Total number of labial papillae [2]	98;100 (88-114)	160 (146-162)	120 (90-144)	168;182 (150-210)
Number of labial papillae per 100 µm [3]	1.5-2	2	2	1.5
Height of exposed part of apical cone cells (µm) [4]	10-12	21-27	35-47	27-32
Number of apical cone cells per 100 µm [4]	10	6-7	6-7	6-7
Number of labial keratodonts per 100 µm [5]	10-14	10-13	6-9	9-12
Number of keratodont serrations [5]	8-10	10-12	12-14	14-16

B. Internal features.

Parameters	*O. oophagus*	*O. taurinus*	*P. resinifictrix*	*P. venulosa*
Total number of postnarial papillae	3-4	3-4	3-4	3-4
Number of lateral ridge papillae per side	1	1	1	1
Number of buccal roof arena papillae per side [6]	(30-45)	40-50	(25-35)	50-60
Diameter of secretory pits in dorsal velar glandular zone (µm)	15-20	15-20	5-15	10-25
Angle between longitudinal axis of nares and transversal body axis	40°-45°	40°-45°	30°-45°	10°-20°
Number of praelingual palps per side	1	1	1	1
Total number of lingual papillae	2	2	2	2
Number of marginal buccal floor arena papillae per side	3-6	11-16	6-10	10-15
Number of central buccal floor arena papillae per side [6]	(13-15)	13-15	(0-3)	12-14
Diameter of secretory pits in ventral velar glandular zone (µm)	20-30	15-30	5-10	15-40
Folding pattern of filter rows [7]	2° [3°]	3° (4°)	2° (3°)	4°
Branching pattern of gills [8]	3°	3°	2°	3°
Length of gill tufts (µm) [8]	350-400	400-500	300-350	450-650
Extension of lungs [9]	$^1/_2$	$^2/_3$	$^3/_4$	$^3/_4$

1. References according to Table I.
2. Median values, ranges in parentheses.
3. In ventromedian position on oral disk.
4. In median position on upper jaw sheath.
5. In median position on continuous upper tooth row.
6. Indistinctly developed papillae in parentheses.
7. Secondary, tertiary, quaternary, features found in some cases in parentheses, found exceptionally in brackets.
8. In median position on second ceratobranchial.
9. Mean maximum caudal extension of inflated lungs in the abdominal cavity.

restricted to specimens of stages 38 ± 2. We excluded material below developmental stage 26 from subsequent statistical analysis because of small sample sizes.

For examination of buccopharyngeal features, scanning electron microscopy (SEM, two specimens per species) and stereo light microscopy (methylene blue staining, three specimens per species) were used. Data on external features were based on stereo microscopic examination. Oral disk structures were confirmed by SEM (two specimens per species). Preparation for SEM examination (Jeol JSM-35 CF) followed a standard procedure (ethanol dehydration, critical-point-drying, gold sputter surface-coating). Measurements were determined using a digital display length-measuring unit (Wild MMS 235) attached to a stereo microscope (Wild M8).

For statistical analyses, SPSS for Windows (Version N° 5.0.2.) was used. Gross body proportions were described by basic descriptive statistics. Since material examined included a wide range of developmental stages, we tested for covariation of ratios (Table III) with total length (univariate regression for each species) and correlation of ratios with stage (Spearman rank correlation), as well as for isometry of growth. Gross body measures were log-transformed and regressed against total length ($ln\ y = ln\ a + b\ ln\ x$; GOULD, 1966). Where the regression coefficient (b) was significantly different from 1.0, the isometry hypothesis (H_0) was rejected. We also estimated the regression of the ratios describing the gross body proportions on total length.

With discriminant analysis, we estimated the optimum linear combination of variables for differential diagnosis and the probability of misclassification of individuals. We used the ratios of gross body proportions for these analyses and included the number of upper and lower tooth rows. The latter two are meristic characters, and number of upper tooth rows did not show any variation in three of the species studied. Therefore, some within group variance-covariance matrices were singular. Discriminant analysis is fairly robust against this type of violation of assumptions, and, as an exploratory tool, provided concise results.

To estimate the relative influences of phylogenetic relationship (genus) and contemporary larval habitat (ecotype), univariate as well as multivariable analyses of covariance (ANCOVA, MANCOVA) were performed (stages 38 ± 2). The dependent variables were logarithms of absolute gross body measures and of numbers of upper and lower tooth rows. A full factorial mixed model was used with ecotype (phytotelm versus pond) and genus (*Osteocephalus* versus *Phrynohyas*) as factors and total length as covariate (JOHNSON & WICHERN, 1988; MORRISON, 1990).

For the material examined, each ecotype was represented in each genus, hence, all cells of the model were occupied. However, the number of individuals in each cell was unbalanced, and the individual sums of squares did not add up to the total sum of squares in the ANCOVA. Since our design is "not extremely unbalanced" (SHAW, 1987), results are not substantively compromised, and the application of other estimators will compare to the ANOVA estimator (SWALLOW & MONAHAN, 1984; SHAW, 1987).

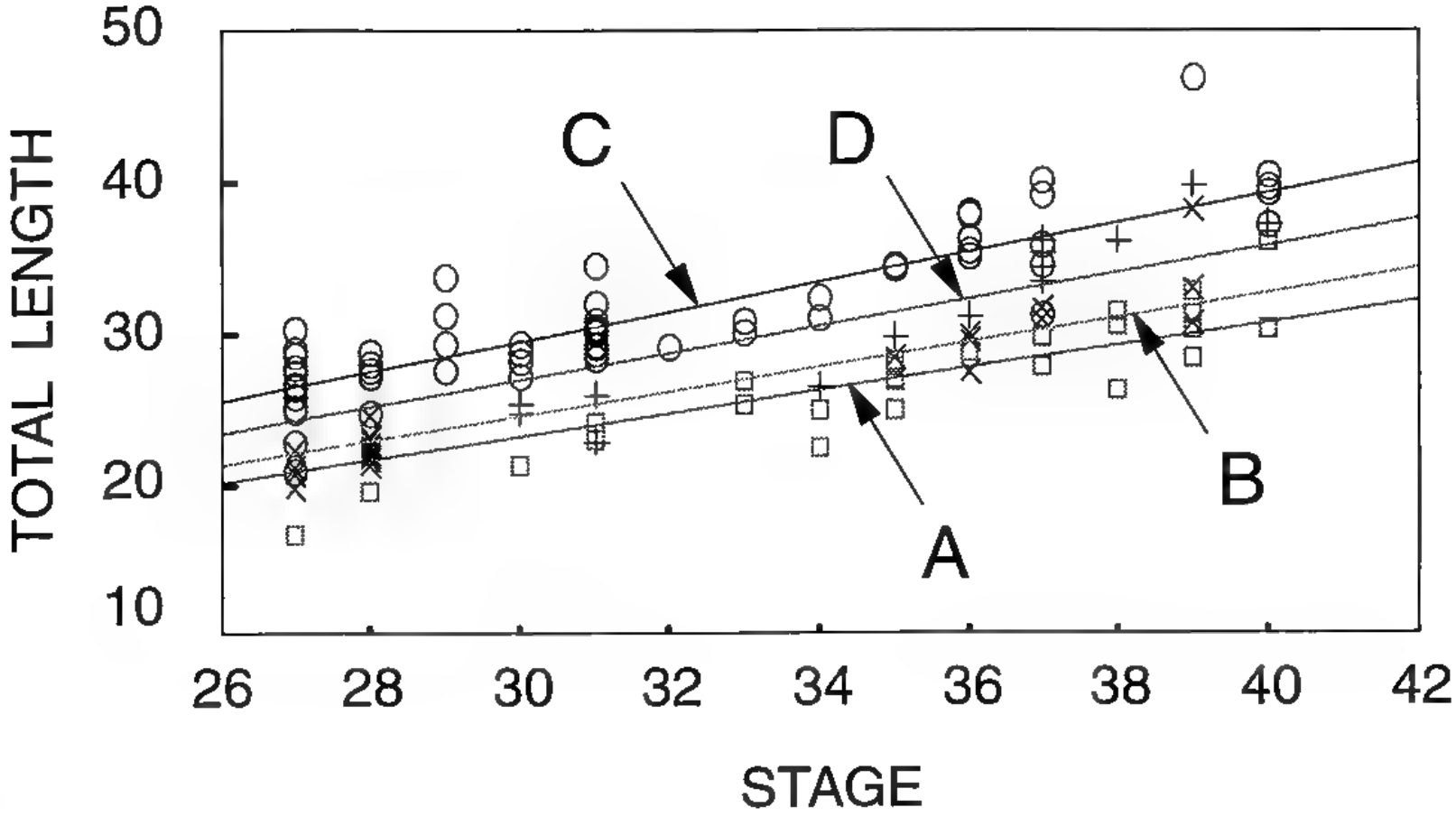


Fig 1 – Size-stage graphs (scatter plots, regression lines) of developmental stages (GOSNER, 1960) and total lengths (mm): *Osteocephalus oophagus* (A, squares), *Osteocephalus taurinus* (B, multiplication signs); *Phrynohyas resinifictrix* (C, circles); *Phrynohyas venulosa* (D, addition signs).

RESULTS

For complete and concise description, presentation of results is based on the following conventions:

(1) Descriptions apply to all four species where no interspecific differences are indicated.

(2) Relative descriptive terms without quantification or reference of comparison refer to external morphology of "generalized pond-type hylid larvae" (DUELLMAN, 1970) and to the terminology for the description of buccopharyngeal morphology as used by, e.g., WASSERSUG (1980), VIERTEL (1982), INGER (1985), and WASSERSUG & HEYER (1988).

(3) Precise values of the ratios describing body proportions and corresponding abbreviations are presented in Table III, metric and meristic values of oral disk and buccopharyngeal features in Table V.

For stages 26 through 40, almost all ratios describing gross body proportions covaried with neither total length nor stage (Table III), and therefore were pooled, in addition to the presentation for tadpoles in advanced developmental stages (38 ± 2). In test for isometry, the regression coefficients did not differ significantly from 1.0, i.e., tadpoles' growth was isometric from stage 26 on in all gross body measures, except for tail height in *O. oophagus* and *P. resinifictrix*. In these species, the tail height grew proportionately less than total length.

Through all stages examined, mean total length increased from *O. oophagus*, through *O. taurinus* and *P. venulosa*, to *P. resinifictrix* (fig. 1).

GENERAL DESCRIPTION

Body slightly depressed; in dorsal view, elongate elliptical in *P. venulosa*, elongate ovoid in *O. taurinus*, round ovoid to piriform in *O. oophagus*, elongate elliptical to piriform in *P. resinifictrix*. In *P. resinifictrix*, *O. oophagus* and *O. taurinus*, body width correlated with amount of ingested eggs. Those eggs were visible through the abdominal wall, notably in *O. oophagus*, whose integument was comparatively pale, thin and transparent.

Spiracular tube sinistral, directed posterodorsally, tightly attached to the body, opening slightly below midline of body, at about one-half to two-thirds of distance from tip of snout to opening of vent tube (SS/SV). Vent tube of moderate size, opening medially to subdextrally at edge of ventral fin. Tail length equal to three-halves the snout-vent length; tail proportionately shortest in *O. oophagus* and longest in *P. resinifictrix* (VT/SV). Dorsal tail fin extending moderately onto body, inserting one-half to two-thirds the snout-vent length distant from tip of snout; insertion of dorsal tail fin most anterior in *P. venulosa* (SV/SU). Tail length twice to four times the tail height; tail height three to four times the height of dorsal tail fin; *O. taurinus* with least maximum tail height in relation to tail length and shallowest upper tail fin compared to the maximum height of tail (VT/HT, HT/UF). Fin edges arched, compared to the margins of caudal musculature,

more convex in *P. venulosa* than in *P. resinifictrix*, fairly parallel in *Osteocephalus*. Upper and lower fins almost equally high (UF/LF), gradually tapering. Caudal musculature moderate, nearly reaching the obtusely pointed tip of tail.

In dorsal view, snout nearly truncate in *O. oophagus*, bluntly rounded in the other species. In lateral profile, snout acutely rounded in *O. oophagus*, rounded in *P. resinifictrix* and *P. venulosa*, bluntly rounded in *O. taurinus*. Oral disk subterminal; position correlated with shape of snout (Table V.A); most anterior and, if expanded, partly visible in dorsal view in *O. oophagus*, and increasingly more posterior from *P. resinifictrix* trough *P. venulosa* to *O. taurinus*. Eyes moderately large, situated dorsolaterally; widely spaced, directed laterally, visible in ventral view in *Phrynohyas*, slightly less separated, directed dorsolaterally, not visible in ventral view in *Osteocephalus*. Nostrils rimmed, directed anterolaterally, about midway between pupillae and tip of snout in *Osteocephalus*, slightly more anterior in *Phrynohyas* (RN/NP), internarial distance about two-thirds the interpupillar distance (PP/NN).

ORAL DISK

Oral disk (fig. 2) medium-sized, moderately expanded laterally, slightly trilobate ventrally. Labial papillae average sized; extension of dorsomedian gap about one-fifth of continuous upper tooth row in *O. oophagus*, *P. resinifictrix* and *P. venulosa*, one-third of continuous upper tooth row in *O. taurinus*. In the fully expanded oral disk, marginal labial papillae arranged in a single or alternating double row; submarginal papillae scattered in the lateral oral disk portions, most frequently ventrolaterally, often in lateral continuation of the outermost lower tooth rows. Labial papillae more numerous in *P. venulosa* and *O. taurinus* than in *P. resinifictrix* and *O. oophagus* (Table V.A); denticulate papillae in the lateral portions of the oral disk and in lateral extension of secondary tooth rows frequently present in *P. venulosa*, exceptionally present in *P. resinifictrix*.

In early larval stages, two upper and three lower labial tooth rows present (Table IV, fig. 7). Labial tooth row formula 2/3 retained throughout entire larval period in *O. oophagus*, and occasionally in advanced stages in *P. resinifictrix*; in the other species, additional lower tooth rows appearing with increasing developmental stages (Table IV). One or two additional upper tooth rows developed only in *P. venulosa* (Tables IV and V.A). Maximum total number of tooth rows 5 in *O. oophagus*, 7 in *P. resinifictrix*, 9 in *O. taurinus* and *P. venulosa*.

Morphology of ontogenetically basic upper two and lower three tooth rows quite homogeneous in all species. Outer upper primary row continuous, inner one shortly interrupted medially, with its median ends often covered by the outer tooth row, both coextending far towards the lateral corners of the oral disk. Ridges of lower three primary rows frequently indented medially when not fully expanded; innermost always continuous in *P. resinifictrix*, occasionally with a very narrow median interruption in the other species; outer two always continuous; all three typically of broad and almost equal lateral extension, outermost often shorter than the inner ones in *O. oophagus*.

Tooth rows in excess of the basic 2/3 pattern added centrifugally in the upper (*P. venulosa*) as well as in the lower labium; typically, with broad median interruption in

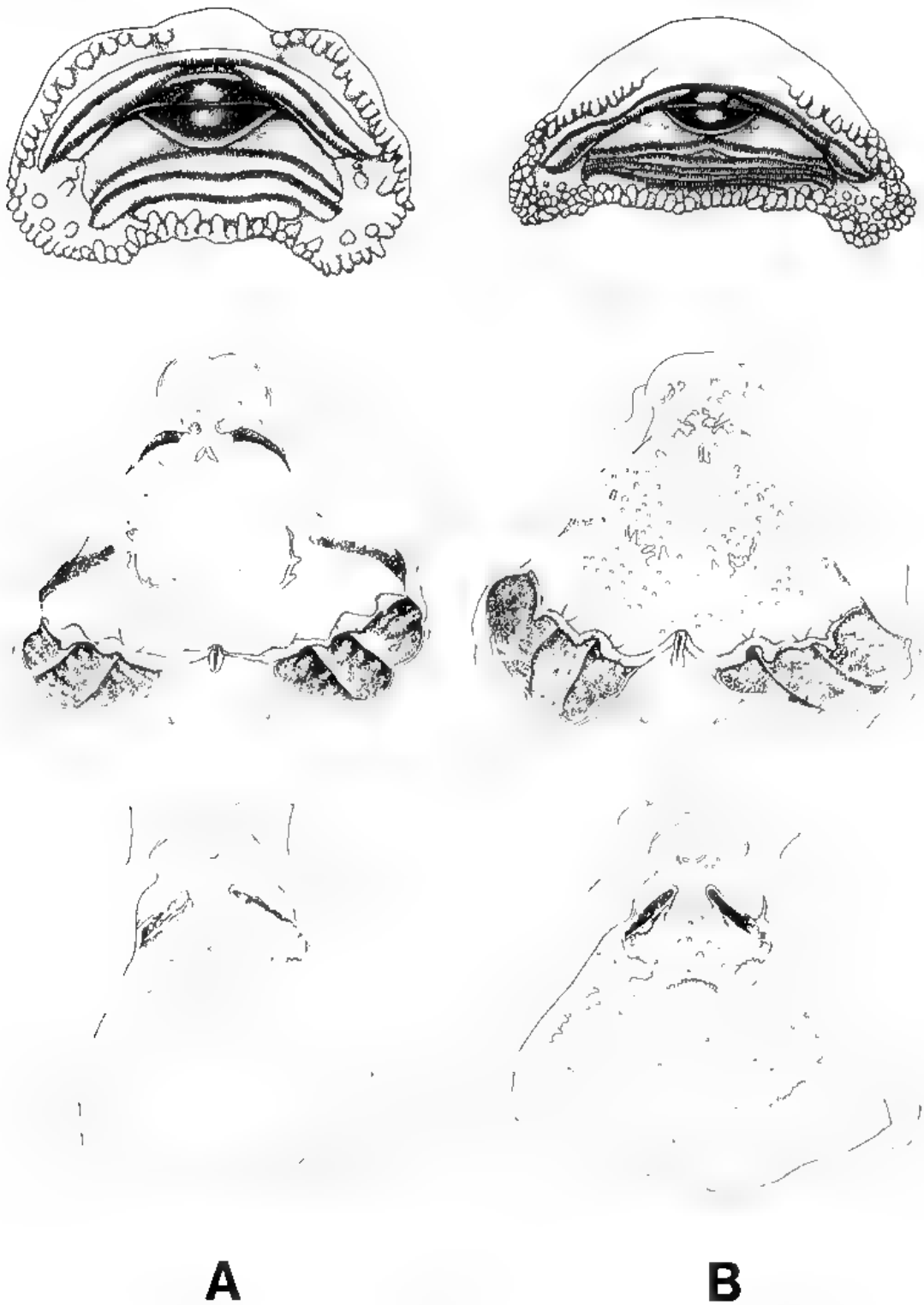
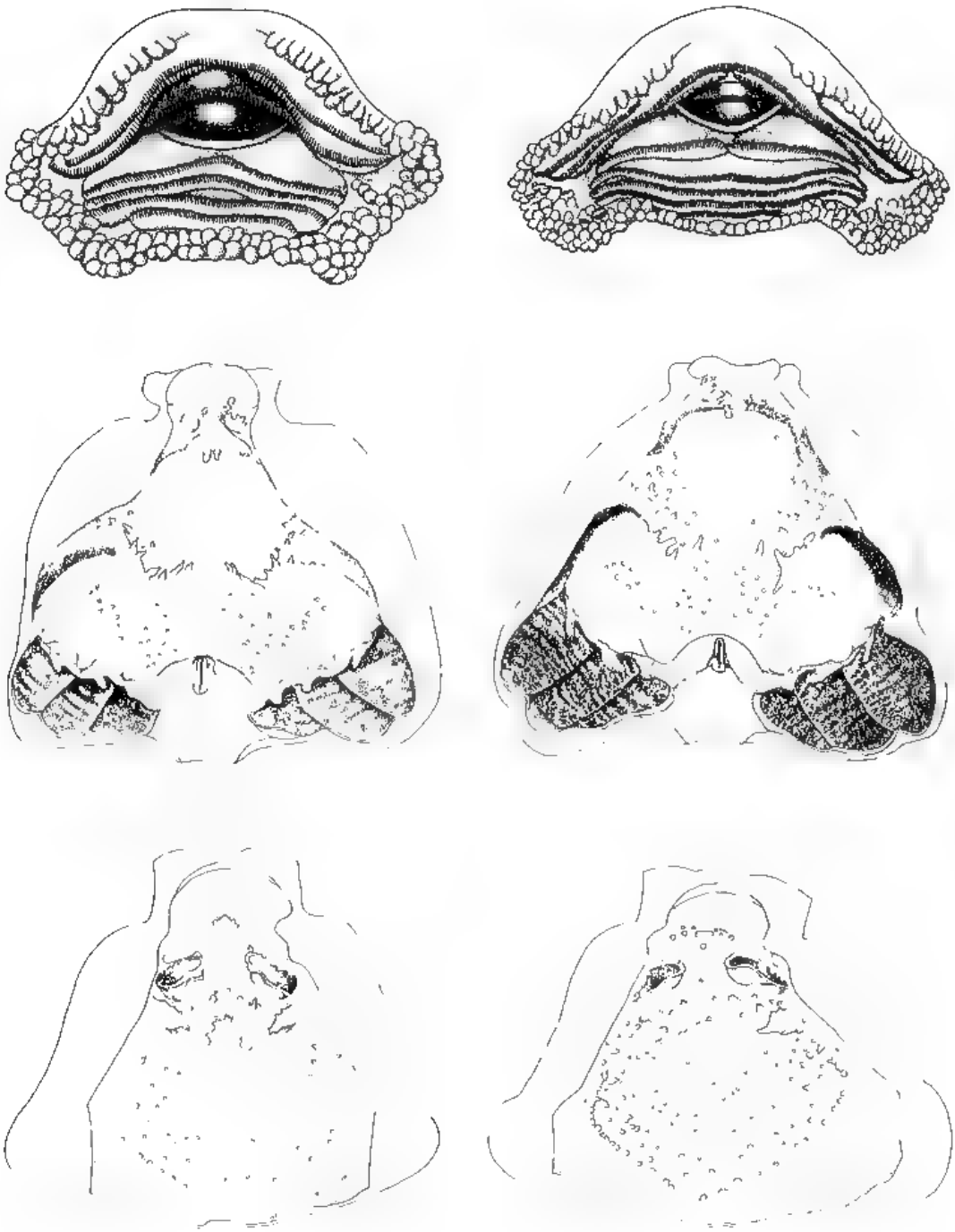


Fig. 2. – Drawings of oral disk (top), floor (middle) and roof (bottom) of buccopharyngeal cavity after SEM micrographs: A, *Osteocephalus oophagus* (1 93/3 87); B, *Osteocephalus taurinus* (2.33/4 94), C, *Phrynohyas resinifictrix* (1.84/5.30); D, *Phrynohyas venulosa* (2 33/4.62). Maxi-



mum horizontal (graph) diameter (in mm) of oral disk / of buccopharyngeal cavity in parentheses. Measures according to SEM micrographs; SEM preparation shrinkage factor about 0.70.

the upper labium (*P. venulosa*), continuous in the lower labium; shorter and more frequently broken the more distally positioned.

Labial keratodonts in a single series on each ridge, cone-shaped with spatulate apical portions bearing acute marginal denticles; size and density of keratodonts, and number of apical indentations slightly varying among species and genera (Table V.A).

Jaw sheaths (fig. 2) broadly dark pigmented, robust, wide, reaching far towards lateral corners of oral disk; front surfaces of average curvature; median part of occlusive margins slightly convex and rectilinear; jaw sheaths moderately narrower and more delicate in *O. oophagus* than in the other species. Edges of upper and lower jaw sheaths smooth in *O. oophagus*, finely serrated in the other species. In SEM examination, exposed parts of apical cone cells incisiviform in *O. oophagus*, caniniform in the other species (fig. 3); shape subrectangular with nearly straight distal edges and tight lateral attachment in *O. oophagus*, acutely pointed, cone-shaped in *O. taurinus*, lanceolate in *Phrynohyas*; longest and most acutely tapered in *P. resinifictrix* (Table V.A).

Buccopharyngeal cavity

Buccopharyngeal surface features quite homogeneous in all four species. Variation mainly restricted to number and size of the papilla-derived structures, being longer and more numerous in *O. taurinus* and *P. venulosa* than in *O. oophagus* and *P. resinifictrix*, with *O. oophagus* showing the simplest and *P. venulosa* the most differentiated pattern (Table V.B, fig. 2).

Buccopharyngeal roof. – Prenarial arena broad, centrally with stout tuberous pustulations, scattered or fused to a median knob or ridge, of variable arrangement even within a species. Internal nares elongate, obliquely oriented; relative length of internal nares in *Phrynohyas* about two-thirds that of *Osteocephalus*; angle between longitudinal axis of internal nares and transversal body axis smaller in *P. venulosa* than in the other species (Table V.B). Anterior narial wall lined by tiny, laterally slightly more elongate papillae; posterior narial wall valve smooth-edged, slightly lobate; narial valve projections faint or slightly lobate. Postnarial papillae arranged in an anteriorly convex arch except for some scattered minor pustulations, well separated from each other in *Osteocephalus*, more basely fused in *Phrynohyas*. One lateral ridge papilla per side, broad-based, palp-like, bearing rather stout or conical pustulations. Median ridge average sized, flap-like; triangular, more slender, elongate, small-based, distant from the lateral ridge papillae, with a pointedly lobed margin in *O. oophagus* and *P. resinifictrix*, semicircular, more stout, broad-based, laterally extended towards the lateral ridge papillae, with the margin tightly bordered by a row of small pustulations in *O taurinus* and *P. venulosa* (Table V.B). Papillae in the spacious buccal roof arena comparatively small in all species. Papillae bordering the arena more distinct; in the lateral corners of the arena slightly elongate in *O. taurinus* and *P. venulosa*, almost absent in *O. oophagus* and *P. resinifictrix*. Lateral roof papillae scarce but minor pustulations. Dorsal velum continuous across midline with the medial edge bare of papillation. Glandular zone distinct in all species. Width of glandular zone and diameter of secretory pits less in *P. resinifictrix* than in the other species (Table V B, fig. 2)

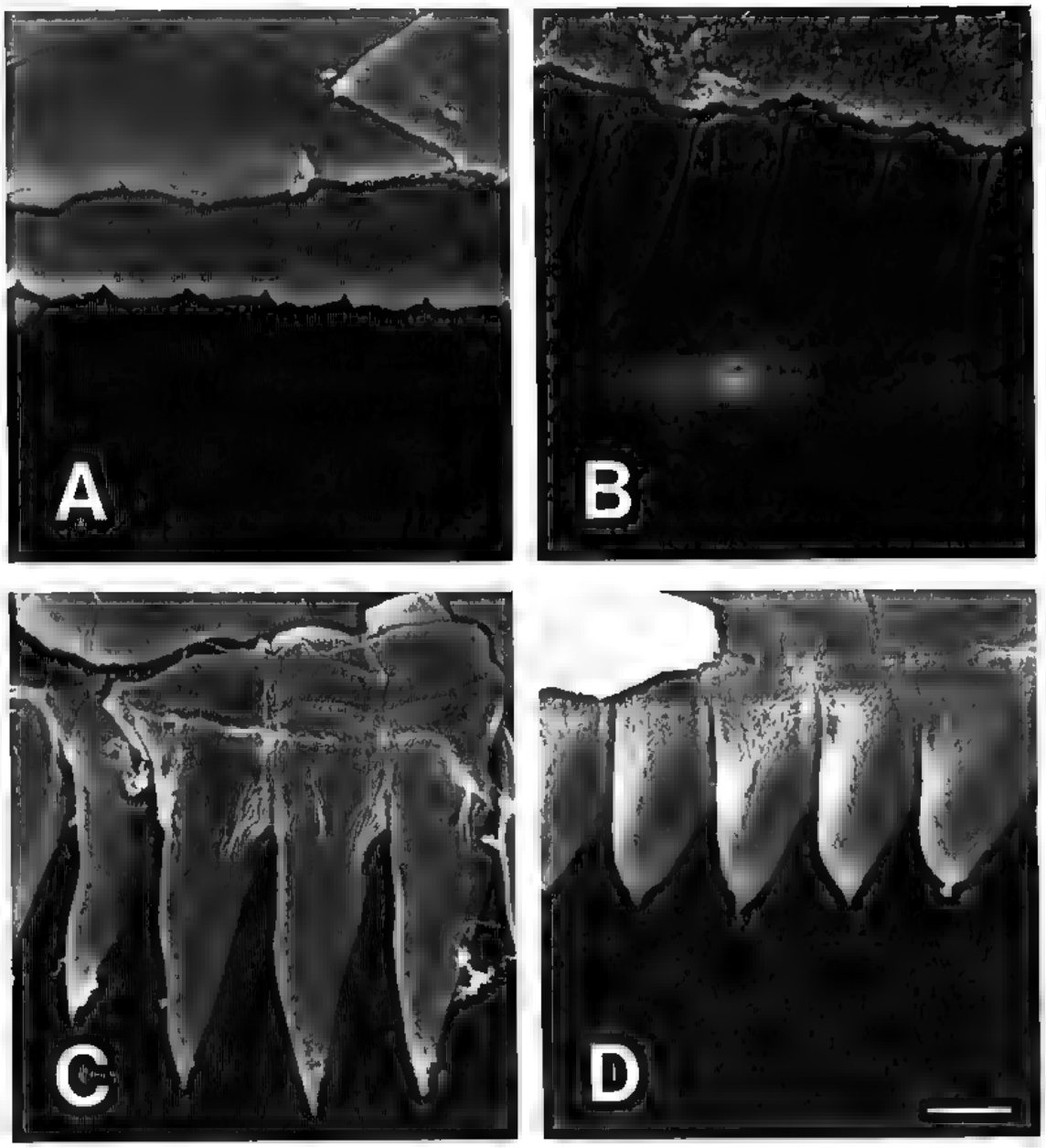


Fig. 3. – SEM micrographs of edge of median upper jaw sheath (scale line equals 10 µm): A, *Osteocephalus oophagus*; B, *Osteocephalus taurinus*; C, *Phrynohyas resinifictrix*, D, *Phrynohyas venulosa*

Buccopharyngeal floor. – Prelingual arena scattered with more or less papilliform, stout, laterally and posteriorly more frequent pustulations; almost bare in *O. oophagus*. One average sized, broadly based prelingual palp per side; somewhat larger with the papilliform marginal lobations more elongate, finger-like, and less numerous in *O. oophagus* and *P. resinifictrix* than in the other species. One pair of slim cylindrical lingual papillae. Buccal floor arena well defined, center almost bare; papillae bordering the arena moderately enlarged, conical, simple; lateroposteriorly most distinct and frequently fused basely.

Buccal pockets large; orientation almost transversal in *O. oophagus*, oblique in the other species. Prepocket papillae scarce stout pustulations. Ventral velum distinct with evident spicular support; three spiculae per side. One marginal velar projection per filter cavity; least developed in *O. oophagus*. Free edge of ventral velum lined by a distinct glandular zone; secretory pits largest in *P. venulosa*, slightly smaller in *Osteocephalus*, less prominent in *O. oophagus* than in *O. taurinus*, considerably smaller in *P. resinifictrix* (Table V.B). Branchial food traps with distinct secretory ridges.

Median notch broad, leaving glottis fully exposed; glottal lips broad, elevated; exposure of glottis less distinct in *O. oophagus* than in the other species (fig. 2). Lungs well developed in all species (Table V.B, fig. 5) already in early ectotrophic stages; almost extending to caudal curvature of abdominal cavity except for *O. oophagus* (Table V.B). Esophageal funnel spacious.

Depth of branchial baskets and complexity of the filter rows – i.e., degree of branching (Table V.B), height, depth, and density of filter rows (fig. 4) – decreasing from *P. venulosa* through *O. taurinus* to *P. resinifictrix* and *O. oophagus*. Internal gills least developed in *P. resinifictrix*, next least in *O. oophagus*, most differentiated in *P. venulosa* (Table V.B, fig. 4).

Discriminant analysis

Discriminant analysis resulted in three, linearly independent, discriminant functions (Table VI). The first discriminant function was dominated by the number of upper tooth rows. The second discriminant function was determined by the ratio of length of tail to height of tail (VT/HT), but was also influenced by the ratio of length of tail to snout-vent length (VT/SV) and the number of tooth rows in the lower labium. The latter two characters also dominated the third and least significant discriminant function. The ratios of height of tail to height of the upper fin (HT/UF), height of the upper fin to height of the lower fin (UF/LF), and of snout-vent length to the distance between tip of snout and insertion of the upper fin (SV/SU) poorly discriminated the species (Table VI.A).

Misclassification of specimens into species was remarkably rare and restricted to the phytotelmonous species (Table VI.B). The group centroid of *P. venulosa* was clearly separated on discriminant function one. On discriminant function two, group centroids were highly positive in *O. taurinus*, while they were negative in *O. oophagus* and *P. resinifictrix*. On the last discriminant function, group centroids of *O. oophagus* and *P. resinifictrix* were distinctly separate. In summary, the phytotelmonous species clustered closely, while the two pond types formed two more separated clusters (Table VI.C, fig. 6).

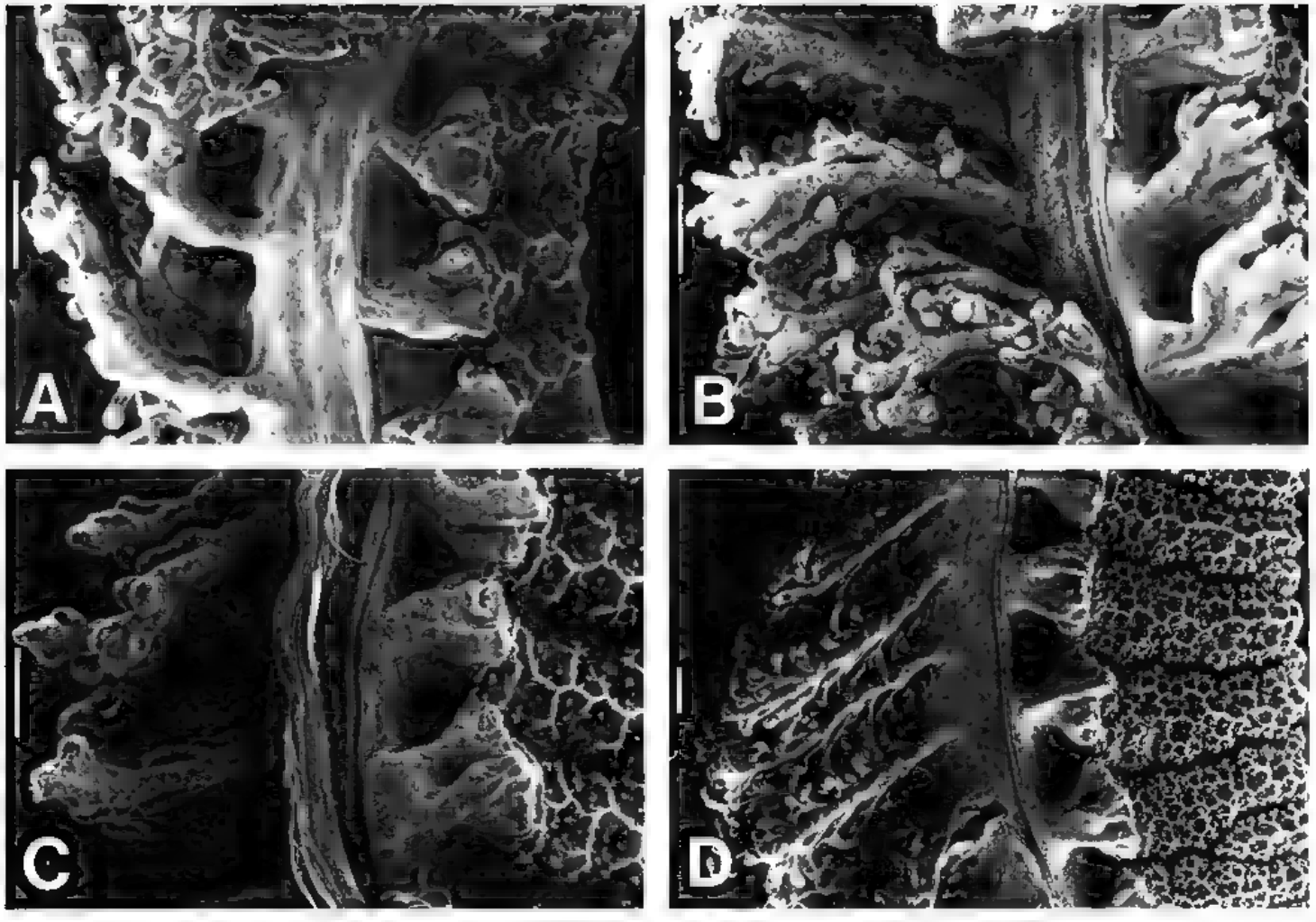


Fig. 4. SEM micrographs of internal gills and filter of median part of second ceratobranchial (scale line equals 100 μm): A, *Osteocephalus oophagus*; B, *Osteocephalus taurinus*; C, *Phrynohyas resinifictrix*; D, *Phrynohyas venulosa*.

Fig 5. — SEM micrograph of floor of the buccopharyngeal cavity and lung sacs of *Phrynohyas venulosa* (scale line equals 1000 µm)

Table VI. - Discriminant analysis of variables describing gross body proportions (abbreviations according to Table III), including number of tooth rows in the upper and lower labium, for *Osteocephalus oophagus*, *Osteocephalus taurinus*, *Phrynohyas resinifictrix* and *Phrynohyas venulosa* (stages 38 ± 2).

A. Coefficients of standardized canonical discriminant functions.

Parameters		Function 1	Function 2	Function 3
Eigenvalues		13.838	3.470	1.576
Vent - tail tip / snout - vent	VT/SV	0.001	-0.602	1.018
Vent - tail tip / tail height	VT/HT	0.103	1.131	-0 265
Tail height / upper fin height	HT/UF	-0 080	-0 209	0.189
Upper fin height / lower fin height	UF/LF	-0.079	-0.203	-0.103
Snout - vent / snout - upper fin	SV/SU	0.080	0.021	-0.012
Number of upper tooth rows	UTR	0.978	-0.010	-0.057
Number of lower tooth rows	LTR	0.075	0.527	0.496

B. Classification of individuals into species (correctly classified individuals on the main diagonal)

Species	Actual numbers of cases	Assigned numbers of cases			
		O. oophagus	*O. taurinus*	*P. resinifictrix*	*P. venulosa*
O. oophagus	20	19	0	1	0
O. taurinus	29	0	29	0	0
P. resinifictrix	43	1	0	42	0
P venulosa	12	0	0	0	12

C. Canonical discriminant functions at group centroids.

Species	Function 1	Function 2	Function 3
O. oophagus	-1.569	-1.243	-2.320
O. taurinus	-1.094	2.886	0 036
P. resinifictrix	-1.347	-1.319	1 077
P. venulosa	10 090	-0.194	-0.076

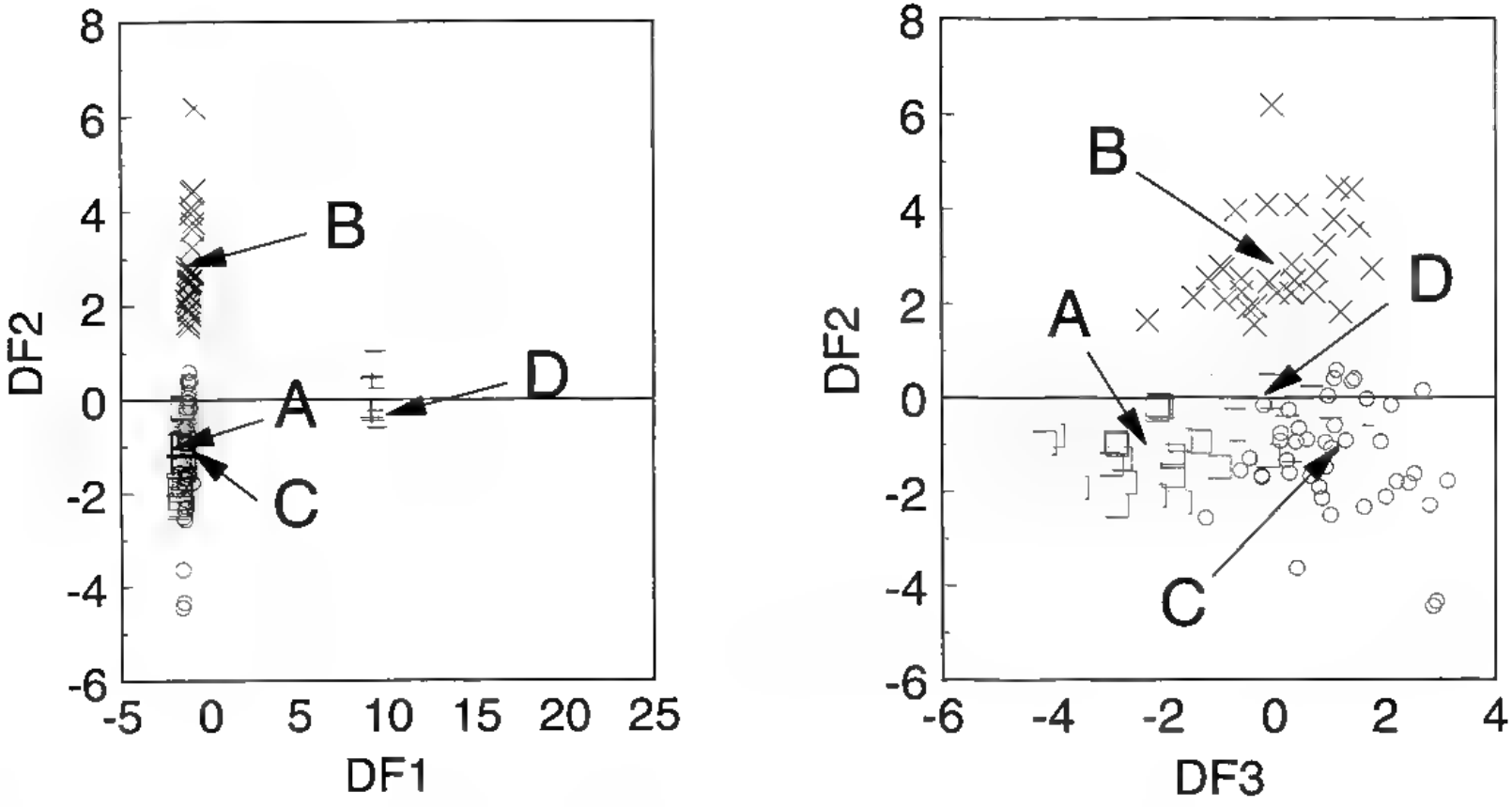


Fig. 6. – Scatter plot of scores of specimens and group centroids (arrows) on discriminant functions (DF) 1 and 2, and 3 and 2: *Osteocephalus oophagus* (A, squares); *Osteocephalus taurinus* (B, multiplication signs); *Phrynohyas resinifictrix* (C, circles); *Phrynohyas venulosa* (D, addition signs).

Table VII. - Analysis of covariance of variables describing gross body proportions (abbreviations according to Table III), including number of tooth rows in the upper and lower labium, for *Osteocephalus oophagus*, *Osteocephalus taurinus*, *Phrynohyas resinifictrix* and *Phrynohyas venulosa* (stages 38 ± 2).
ANCOVA: sums of squares (* $P < 0.05$, ** $P < 0.01$; individual sums of squares do not sum to total sum, because occupation of cells is unbalanced).
MANCOVA: coefficients of standardized canonical discriminant functions.

Parameters		ANCOVA							MANCOVA		
		Total length	Genus	Ecotype	Inter-action	Ex-plained	Residual	Total	Genus	Ecotype	Inter-action
Snout - vent	SV	0 16**	0 00	0.02**	0 02**	0.30**	0 08	0 38	0 121	0 378	-0 387
Tail height	HT	0 07**	0 33**	0 13**	0 16**	1 16**	0 15	1 30	-0 522	0 535	-0 444
Snout - upper fin	SU	0 18**	0 25**	0 08*	0 07*	0 48**	0 40	0 89	0 121	-0 091	0 110
Number of upper tooth rows	UTR	0 00	0 28**	0.48**	0 41**	1 27**	0.07	1 34	-0 737	-0 678	-0 661
Number of lower tooth rows	LTR	0.00	0 01	1 52**	0 44**	2.26**	0 28	2 26	-0 009	-0 718	0 609

ANALYSIS OF COVARIANCE

With ANCOVA, influence of total length, ecotype, genus, and the interaction between ecotype and genus, were significant for gross body measures with the exception of genus on snout-vent length. The tooth row characters were not influenced by total length; influence of ecotype and the interaction between ecotype and genus were significant for both upper and lower tooth rows, whereas the influence of genus was only significant for the number of upper tooth rows (Table VII).

MANCOVA produced three, linearly dependent, discriminant functions (Table VII). Both ecotypes and genera were best differentiated by number of upper tooth rows but also by height of tail. Number of lower tooth rows only differentiated between ecotypes. For the effect of genus, tooth row characters and height of tail enter with identical signs, whereas, for the effect of ecotype, these variables enter with opposite signs. Interactions were influenced mainly by the tooth row characters with opposite signs, but also by height of tail and snout vent length, both in the same direction with the number of upper tooth rows. Craniad extension of upper fin discriminated only weakly between any factor or interaction (Table VII).

The observed standardized MANCOVA discriminant functions associated with genus, ecotype, and their interaction, condensed the morphometric results. compared with the pooled pond-dwelling species, the pooled phytotelmonous species were characterized by fewer upper and lower tooth rows, while, relative to their total length, they had higher tails. For the pond-dwelling species, higher number of lower tooth rows was found in both genera while higher number of upper tooth rows was found only in *P. venulosa*.

Discrimination in height of tail between ecotypes and genera was influenced mainly by *O. taurinus*, which had the relatively lowest tail, while the tail characteristics of the other species were comparatively uniform.

Discussion

Tadpoles of both the pond-dwelling and the phytotelmonous species studied are characterized by overall average body dimensions and, thus, resemble typical hylid pond tadpoles (Duellman, 1970). *P. resinifictrix* and *P. venulosa* larvae are longer, have proportionately longer tails, higher upper tail fins, more lateral eyes, and more anterior nares than *O. oophagus* and *O. taurinus* larvae. As far as known from the literature (see caption of fig. 7), these intergeneric differences in height of tail and position of eyes and nares also apply to the other species of the two genera.

Collectively, buccopharyngeal features of the four species studied represent a relatively uniform type, which we consider omnivorous to macrophagous and capable of both branchial and pulmonary respiration. Nevertheless, number and size of buccopharyngeal papillae, complexity of branchial filter system, development of velar secretory tissues, differentiation of gills, along with number of tooth rows and of labial papillae, correspond to the principal larval habitat types as usual among anuran larvae: relative structural "simplification" characterizes the phytotelmonous larvae, wheras "elaboration" characterizes the pond-dwelling ones (Wassersug, 1980; Altig & Johnston, 1989).

Less differentiated external and internal buccopharyngeal features along with the more anterior position of the oral disk in the phytotelmonous species are explained by their predominantly macrophagous feeding habits. Among the phytotelm-dwelling species analyzed, however, most internal buccopharyngeal features are less differentiated in *O. oophagus* than in *P. resinifictrix*. This divergence may be explained by different degrees of "specialization" (Trueb, 1973) to macrophagous nutrition: *O. oophagus* is obligatorily macrophagous, feeding on conspecific fertilized eggs and tadpoles (Hödl, 1993), while *P. resinifictrix* is omnivorous (Grillitsch, 1992; Schiesari, 1993), predominantly macrophagous (Schiesari, 1993), feeding mainly on conspecific fertilized eggs, but also on detritus (Schiesari & Gordo, 1993). Among the pond-dwelling species, buccopharyngeal surface features are more differentiated in *P. venulosa*, indicating a greater reliance on microphagous feeding than in *O. taurinus*. *O. taurinus* larvae have been observed in the field to be voracious egg-eaters (Schiesari, personal observation), which matches the morphological indication of macrophagy. *Osteocephalus elkejungingerae*, whose tadpoles are highly cannibalistic when laboratory bred (Henle et al., 1983), represents a further species within the genus with macrophagous larvae.

Lungs are spacious in the four species analyzed, although more expanded in *Phrynohyas* than in *Osteocephalus*. Comparing among phytotelmonous larvae, glottis and lungs are large in *P. resinifictrix* as in the tree hole-dwelling *Philautus* sp. and *Theloderma stellatum* (Wassersug et al., 1981), but are medium sized in *O. oophagus* as in the bromeliad-dwelling *Osteopilus brunneus* (Lannoo et al., 1987). Among the two types of

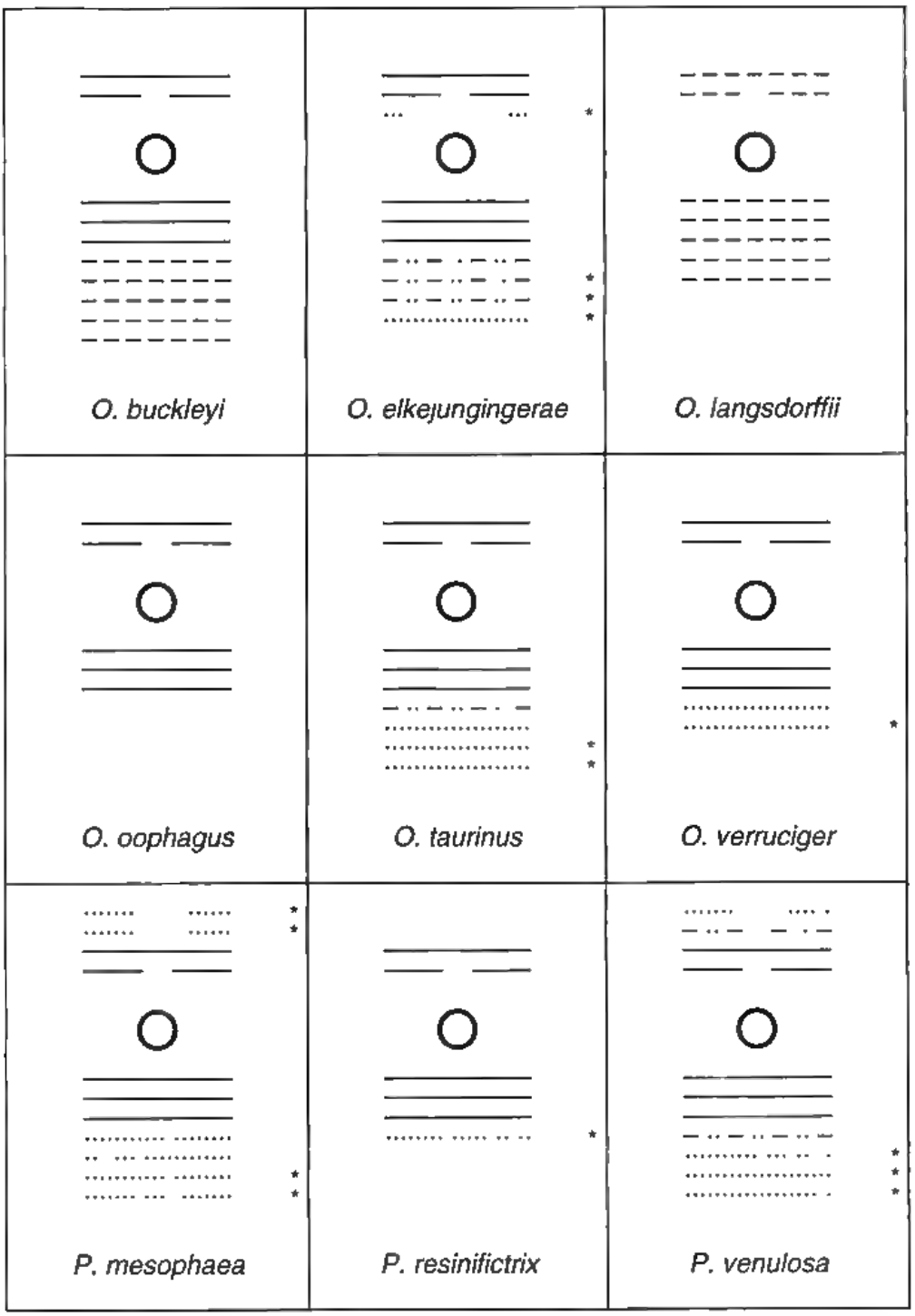


Fig 7. Variation of larval tooth row formulae among *Osteocephalus* and *Phrynohyas* species Schematic drawings; median interruptions and relative lengths of lower tooth rows not considered

References: *Osteocephalus buckleyi* (HERO, 1990); *Osteocephalus elkejungingerae* (HENLE, 1981); *Osteocephalus langsdorffii* (DUELLMAN, 1974); *Osteocephalus oophagus* (present study and as in Table I); *Osteocephalus taurinus* (present study and as in Table I), *Osteocephalus verruciger* (TRUEB & DUELLMAN, 1970); *Phrynohyas coriacea* (SCHIESARI & MOREIRA, in press); *Phrynohyas mesophaea* (LUTZ, 1973; SCHIESARI, personal observation); *Phrynohyas resinifictrix* (present study and as in Table I); *Phrynohyas venulosa* (present study and as in Table I).

phytotelms, tree holes offer the more anaerobic aquatic environment: dissolved oxygen was 0.2 mg/l (surface water 26°C, 25 l water volume) in tree hole water dwelled by *P. resinifictrix* larvae, but 2.6 mg/l (26°C, 10-15 ml water volume) in the water of bromeliad leaf axils inhabited by *O. oophagus* larvae (SCHIESARI, personal observation). Correspondingly, development of lungs indicates greater importance of pulmonary respiration in tree hole-dwelling larvae than in the bromeliad-dwelling ones, whereas gill development indicates that the contrary applies to branchial respiration. Comparing within a genus, development of internal gills and lungs indicate greater reliance on branchial respiration but also on pulmonary respiration (especially in *O. taurinus*) in the pond-dwelling species. "Internal oral structures of anuran larvae can be used to make reasonably sound predictions about the feeding and respiratory ecology of anuran larvae" (WASSERSUG, 1980). Respiratory structures in the species studied indicate comparatively low average levels of dissolved oxygen also in the larval pond habitats. However, since "lungs appear to be advantageous to aquatic organisms even in normoxic water in that they allow buccopharyngeal surfaces to be dedicated fully to feeding rather than respiration" (WASSERSUG & MURPHY, 1987), extensive development of lungs in the pond-dwelling species studied might further correlate with their less macrophagous, more omnivorous nutrition and, at last, with correspondingly higher motility and metabolic rate in these species which, in contrast to the phytotelmonous ones, develop without "parental" food supply in a, typically, less confined habitat.

For the four species studied, shape of labial keratodonts represents a type very common in Ranoidea tadpoles (e.g., HÉRON-ROYER & VAN BAMBECKE, 1889; GOSNER, 1959; INGER, 1985; ALTIG & JOHNSTON, 1989). Gross jaw sheath morphology shows no considerable peculiarities in the pond-dwelling larvae but is remarkable in the phytotelmonous ones (e.g., DUELLMAN, 1970): edges are smooth in *O. oophagus*, whereas *P. resinifictrix* shows elongate, acutely pointed serration. Smooth edged jaw sheaths are rare in anuran larvae. Among phytotelmonous tadpoles, the upper jaw sheath is smooth and the lower finely serrated in two oophagous species, the bromeliad-dwelling *Hyla zeteki* (DUELLMAN, 1970: 326, first paragraph) and the tree hole-dwelling *Philautus* sp. (WASSERSUG et al., 1981); furthermore, in some egg-eating Jamaican hylids, jaw sheaths are not denticulate (NOBLE, 1929). However, some stream-dwelling tadpoles (*Hyla mixe*, *Hyla mixomaculata*) also bear smooth-edged jaw sheaths (DUELLMAN, 1970), and, in contrast, fine uniform jaw sheath serration is frequently reported for phytotelmonous, oophagous larvae (e g., *Osteopilus brunneus*, LANNOO et al., 1987; *Theloderma stellatum*, WASSERSUG et al., 1981). Thus, various jaw sheath patterns are apparently suitable for oophagous feeding. Smooth-edged jaw sheaths are likely to have different functional correlates in rheophilous and oophagous tadpoles. In rheophilous larvae, smooth jaw sheaths may be most effective for grazing on constrained epilithic substrates; in obligatorily macrophagous oophagous larvae, such as *O. oophagus*, serration simply may have become unnecessary or even disadvantageous for ingesting eggs as a whole.

In the ontogenetic sequence of tooth row appearance, the labial tooth row formula 2/3 is primary in both genera (fig. 7). Additional, secondary upper tooth rows develop in *Phrynohyas* (except in *P. resinifictrix*), where they are added distally. In contrast, they are absent in *Osteocephalus*, with the exception of *O. elkejungingerae*, where they are proximal and poorly formed. Hence, the two genera are well distinguished by different derived types

of ontogenetic tooth row increase (types A to E in ALTIG & JOHNSTON, 1989): in both genera, tooth rows are added centrifugally in the lower labium, but addition of tooth rows in the upper labium is absent (type not considered in ALTIG & JOHNSTON, 1989) or centripetal (type B) in *Osteocephalus* and centrifugal (type C) in *Phrynohyas*. The basic 2/3 tooth row pattern (type A) persists only in the phytotelmonous *O. oophagus* and occasionally in *P. resinifictrix*.

Total numbers of tooth rows of 5 or more than 5, as in *O. oophagus* and in *P. resinifictrix* respectively, compare to the highest known for phytotelmonous hylids (LANNOO et al., 1987). Likewise, among non-phytotelmonous hylids and anuran larvae in general (ALTIG & JOHNSTON, 1986, 1989), total number of tooth rows is comparatively high in all *Osteocephalus* and *Phrynohyas* species (fig. 7): in the pond- and stream-dwelling *Osteocephalus* species, maximum total numbers of tooth rows vary from 7 to 10, which is typical for lotic but not rheophilous hylid tadpoles. Surprisingly, the highest tooth row counts of up to 10 or 11 are present in the evidently lentic *Phrynohyas coriacea*, *P. mesophaea* and *P. venulosa*, as in the pond-dwelling tadpoles of the hylid *Trachycephalus jordani* (MCDIARMID & ALTIG, 1990). These tooth row counts exceed the upper limit of the range of variation known for other lentic hylids, and, furthermore (compared to the data in ALTIG & JOHNSTON, 1986), are within the upper third of the range of variation in lotic hylid tadpoles.

Upper and lower tooth row counts are negatively imbalanced in the two genera studied. Balance values of −1 and −2 as shown in *O. oophagus* and *P. resinifictrix* are moderate among phytotelmonous tadpoles (LANNOO et al., 1987), and represent the most frequent type among hylids as well as among anuran larvae in general (ALTIG & JOHNSTON, 1986, 1989). In all other *Osteocephalus* and *Phrynohyas* species, number of tooth rows in the lower labium exceeds that in the upper labium notably (fig. 7): balance values of −3 to −4 are the most common in the two genera (fig. 7) and in *Osteocephalus* even reach −5 (*O. taurinus*) and −6 (*O. buckleyi*). However, in anurans in general, balance values of −3 to −6 are rare and are most common to lotic larvae (usually neotropical *Hyla* species), though −3 may be also found in phytotelmonous larvae (e.g., *Hyla bromeliacea*; DUELLMAN, 1970).

Among hylids, the combination of a wide dorsomedian interruption of the peribuccal papillary margin, as typical for "generalized pond-type" hylid tadpoles (DUELLMAN, 1970), with a number of tooth rows exceeding the typical pond-type 2/3 pattern, is rare and characterizes both *Osteocephalus* and *Phrynohyas*. A wide dorsomedian papillary gap and increased number of lower tooth rows, as is typical in *Osteocephalus*, is known, e.g., in the bromeliad *Hyla dendroscarta* and the pond-dwelling *Hyla rufitela* larvae (DUELLMAN, 1970). A wide dorsomedian papillary gap and increased numbers of both upper and lower tooth rows, as is typical in *Phrynohyas*, is only known in the pond-dwelling tadpoles of *Hyla geographica* (BOKERMAN, 1963; HERO, 1990; RADA DE MARTINEZ, 1990) and *Trachycephalus jordani* (MCDIARMID & ALTIG, 1990). Let us mention here that TRUEB (1970) suggested comparatively close phylogenetic relationship for the genera *Osteocephalus*, *Phrynohyas* and *Trachycephalus*. This proposal based on geographical and adult morphological evidence is supported by larval oral disk morphology and is not contradicted by the other larval features examined in this study.

Summary and conclusions

Although breeding sites of the species studied in depth are assigned to two principal types (phytotelms and ponds), with discriminant analysis, the external morphological characters analyzed cluster the four species into three distinct groups (fig. 6). The first is the phytotelmonous group with only slight differences between the bromeliad species (*O. oophagus*) and the tree hole habitating species (*P. resinifictrix*), suggesting comparatively little ecological diversity among these species. The other two morphotypological groups are both pond forms (*O. taurinus* and *P. venulosa*), suggesting greater ecological diversity in that habitat.

Among phytotelmonous hylids (as reviewed in Lannoo et al., 1987), external larval morphology assigns both *O. oophagus* and *P. resinifictix* to a relatively "generalized" (Trueb, 1973) larval type in that they show "typical pond tadpole" (Lannoo et al., 1987) body proportions, and oral disk features similar to those phytotelmonous species which feed mainly on detritus. For both species analyzed, dietary information and buccopharyngeal morphology indicate predominating oophagous, carnivorous macrophagy, reduced microphagy, and branchial as well as pulmonary respiration with evidently greater reliance on generalized diet in *P. resinifictrix*. For *P. resinifictrix*, Lannoo et al. (1987) therefore stated that "they appear restricted to larger aquatic bodies, which are more likely to occur in tree holes than in leaf axils". *P. resinfictrix*, in fact, is exclusively known to breed in spacious tree holes (Table VIII). Comparatively high degree of morphological congruency of *P. resinifictrix* and *O. oophagus* corresponds to their collectively relatively low degree of "specialization" (Trueb, 1973), which, for *O. oophagus*, may be explained by its remarkable flexibility in breeding habitat selection: although typically breeding in bromeliads, this species has also been reported to breed in other, considerably diverse water-filled plant structures (Table VIII).

For the non-phytotelmonous larvae of the two genera, data from the literature on external larval morphology of other species greatly match the intergeneric differences observed in the species studied in depth in this study. Collectively, comparatively high tail fins, lateral eyes, and balanced tooth row formulae, as in *Phrynohyas*, are typical for nektonic lentic tadpoles, while the opposite, as shown by *Osteocephalus*, is typical for benthic, commonly moderately lotic larvae. Data compiled from the literature on breeding habitats of non-phytotelmonous congeners (Table VIII) apparently parallel the above morphotypological grouping: all *Phrynohyas* species regularly breed in ponds, whereas *Osteocephalus* larval habitats comprise lentic, and facultatively as well as permanently lotic habitats. Most of the *Osteocephalus* species, in fact, breed in a stream habitat.

However, total number of tooth rows in the lentic *Phrynohyas* species matches or exceeds that of the most lotic *Osteocephalus* species, and, thus, corresponds to habitat inversely than usual among anuran larvae. Furthermore, compared to typical pond-type larvae, tooth row counts are unusually high and balance values are unusually low at least in the non-phytotelmonous species of both genera, more like in lotic rather than in lentic tadpoles. Our observations might be paralleled in other groups of neotropical hylids: Wassersug (1980) found a mosaic of stream and pond related features in the

Table VIII. - Literature survey on breeding habitats of the *Osteocephalus* and *Phrynohyas* species with information on larval external morphology available (fig 7) Regions refer to the sites of observation and do not necessarily cover the species' entire range of distribution.

Species	Principal habitat types			Habitats		Regions	References
	Stream	Pond	Phytotelm				
O buckleyi	+	-	-	Small streams	Primary forest	Central and upper Amazon basin (Brasil, Perú)	HERO (1990); HÖDL (1990, 1993); RODRÍGUEZ & DUELLMAN (1994)
O. elkejungingerae	+	-	-	Small, shallow, slowly to moderately fast flowing waters	Forest	Lower eastern Andean slopes (Perú)	HENLE (1981), HENLE et al (1983); HENLE (1992)
O langsdorfii	-	+	-	Temporary ponds	Forest-border	Atlantic forest (Brasil)	DUELLMAN (1974); FROST (1985); SCHIESARI (pers. obs.)
O oophagus	-	-	+	Epiphytic or ground bromeliads, palm leaf axils, palm bracts lying in the ground, tree holes up to about 35 m high, plastic basins on the ground	Primary and secondary forest	Central Amazon basin (Brasil)	JUNGFER & SCHIESARI (1995); JUNGFER & WEYGOLDT (1995)
	-	-	+	Leaf axils in arboreal plants, bromeliads, palm leaf axils, equivalent arboreal plant structures	Forest	Central Amazon basin (Brasil)	HERO (1990), HÖDL (1990, 1993) [1]
O species	-	-	+	Bromeliads	Primary forest	Upper Amazon basin (Perú)	RODRÍGUEZ & DUELLMAN (1994)
O. taurinus	+	+		Large and small, temporary and permanent ponds, streamside ponds and isolated forest ponds, large and small streams, in forest and forest-edge sites	Primary and secondary forest	Central and upper Amazon basin (Brasil, Perú)	BOKERMANN (1964); HERO (1990); HÖDL (1990, 1993); ZIMMERMAN & RODRIGUES (1990), GASCON (1991, 1993); RODRÍGUEZ & DUELLMAN (1994)
O verruciger	+	-	-	Quiet pool in a stream	Humid montane forest	Lower eastern Andean slopes (Ecuador)	TRUEB & DUELLMAN (1970, 1971)
P cornacea	-	+	-	Temporary ponds	Primary forest	Central and Upper Amazon basin (Brasil, Perú)	HÖDL (1990, 1993), RODRÍGUEZ & DUELLMAN (1994); SCHIESARI & MOREIRA (in press)
P mesophaea	-	+	-	Temporary ponds	-	Atlantic forest (Brasil)	FROST (1985); SAZIMA (1974)
P resinifictrix	-	-	+	Spacious cavities high in large trees	Primary forest	Amazon basin (Brasil, Perú)	References in ZIMMERMAN & HÖDL (1983), HERO (1990, 1991); GRILLITSCH (1992), HÖDL (1993); SCHIESARI (1993); RODRÍGUEZ & DUELLMAN (1994)
P venulosa	-	+	-	Shallow temporary ponds	Open, nonforested but also primary and secondary forest sites	Neotropical lowlands	References in ZIMMERMAN & HÖDL (1983), RODRÍGUEZ & DUELLMAN (1994)

1. Described under the name *Osteocephalus* sp (W HÖDL, personal communication)

bromeliad-dwelling *Hyla dendroscarta* tadpoles. Within species, closely related to *H. dendroscarta*, he recognized a variety of breeding habitats, which, as in the genera *Osteocephalus* and *Phrynohyas*, comprise ponds, streams, and phytotelms.

In summary, *Osteocephalus* and *Phrynohyas* larval habitats are notably diverse. But morphologically, larvae among the two genera are less diverse in that they share similar oral disk and buccopharyngeal features as well as overall average body proportions, high number of tooth rows, low balance values, omnivorous to macrophagous diets, and branchial as well as pulmonary respiration. Particularly, number of tooth rows and lung development do not correspond to habitat in the usual anuran larval fashion.

Two, not mutually exclusive, biological explanations for the generally high number of tooth rows and the well developed lungs in the extant non-phytotelmonous species examined are possible.

(1) Adaptation to contemporary environment. – Their contemporary larval environment is collectively characterized by comparatively high temperatures and correspondingly low oxygenation If aerial respiration is the expected major factor in the development of lungs (as reviewed in WASSERSUG & SEIBERT, 1975 and WASSERSUG & MURPHY, 1987), low levels of dissolved oxygen favor early and extensive development of lungs in lentic but also in lotic tadpoles (NOBLE, 1929). If adhesion to substrate is the expected major action among the suggested functions of labial teeth (as reviewed in ALTIG & JOHNSTON, 1989), increased number of tooth rows, i.e., increased adhesive efficiency of the oral disk, might be an adaptation to compensate the hydrodynamic disadvantage (NOBLE, 1929; WASSERSUG, 1980) of well developed lungs especially in lotic environments. This explanation is more likely to apply to the lotic benthic *Osteocephalus* species than to the lentic nektonic *Phrynohyas* species. For both genera, increase in adhesive capacity of oral disk might also be a not yet considered correlate to macrophagous nutrition.

(2) Persistent influence of ancestral patterns. – Some features, such as high number of tooth rows in the lentic species, might represent an ancestral lotic pattern and might have persisted relatively unchanged. If breeding in phytotelms is "derived" (TRUEB, 1973) in the genera studied, other features, such as pulmonary respiration and omnivorous, predominantly macrophagous nutrition in non-phytotelmonous larvae, might further be exaptations (GOULD & VRBA, 1982) to life in lowly oxygenated and "confined" (e.g., LANNOO et al., 1987) phytotelmonous habitats.

RESUMEN

La morfología externa y bucofaringea de larvas habitantes de fitotelmata y charcos de cuatro especies de hílidos neotropicales fueron analizadas con relación a diagnosis diferencial y ecomorfología. Los hábitats larvales tipicamente comprenden bromélias (*Osteocephalus oophagus*), hoquedades en árboles (*Phrynohyas resinifictrix*), charcos en áreas de selva (*Osteocephalus taurinus*) y charcos en áreas abiertas (*Phrynohyas venulosa*). Un análisis discriminante de caracteres externos morfométricos reveló dos subgrupos ligeramente diferentes dentro del grupo de habitantes de fitotelmata pero dos subgrupos bien separados dentro del grupo de los habitantes de charcos. Todas las especies

mostraron proporciones corporales del tipo de un renacuajo generalista, hábitos macrófagos y respiración tanto branquial como pulmonar. Los renacuajos que viven en fitotelmata fueron caracterizados por la reducción de las estructuras peribucales y bucofaringeas, y difirieron marcadamente en la morfología del pico córneo y en el desarrollo de los pulmones. Los renacuajos que se desarrollan en charcos fueron caracterizados por un alto número de filas de dentículos y por valores de balance bajos. Los géneros *Phrynohyas* y *Osteocephalus* se distinguieron mejor por el desarrollo de filas de dentículos superiores secundarias, por la posición de los ojos y por las proporciones corporales groseras.

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Systematics, morphometrics and biogeography of the genus *Aubria* (Ranidae, Pyxicephalinae)

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New data are given on geographic distribution and morphometric variation with reference to geographic origin and sex in *Aubria subsigillata* (Duméril, 1856) and *Aubria masako* Ohler & Kazadi, 1990. The recently described *Aubria occidentalis* Perret, 1995 is here considered as a synonym of the former. Different statistical approaches are used to describe the taxa and the observed variations. A hypothesis of femoral gland function is formulated in the genera *Aubria* and *Pyxicephalus*. A phylogeny of the group *Aubria-Pyxicephalus* (Pyxicephalinae) is discussed.

INTRODUCTION

Naturalists conducting systematics and biogeographical surveys often encounter two patterns of distribution. A species can have a distribution-limited area, with little morphological variation, or a very wide distribution, within which one frequently observes significant variation that may lead to more than one interpretation. Often discrimination of distinct units is not discrete, and taxonomic decision difficult if collection localities are distant from each other. Even when we are conscious of variation problems in biology and use appropriate sampling methods, old samples generally include too few specimens for statistical comparison.

A recent study of frogs of the ranid genus *Aubria* (OHLER & KAZADI, 1990) led us to distinguish a new species (*Aubria masako*) from the central region of Zaire, North of the Congo. This species is clearly distinct from the type-specimen of *Rana subsigillata* from Gabon (DUMÉRIL, 1856). Specimens from 17 other localities of western Africa were referred to the species *Aubria subsigillata* by use of morphometrical (internarial distance, snout-nostril distance, diameter of tympanum) and morphological (presence of femoral glands, presence of mid-dorsal stripe) data. However, it was not possible to assign three frogs in the Paris Museum collection from the Sangha region, because they showed characters which distinguished them from our samples. The collection of further data was needed to resolve the question of their relationships.

Another problem presented by the literature was the presence of femoral glands in some of the frogs of populations without mid-dorsal stripes and their absence in others (DE

WITTE, 1930, PARKER, 1936; PERRET, 1966). PERRET (1966) had indicated that variation exists in this character among the frogs of Cameroon, and recently (1995) he discussed variation of this character in relation to sex. Finally, according to AMIET (personal communication), in Cameroon there are two different mating calls in *Aubria*, characterizing two kinds of frogs that are also distinct in distribution and ethology.

Recently, I collected important additional information on frogs of the genus *Aubria*. This gives a more detailed knowledge of the distribution of these frogs, contributes to the biogeography of Africa, and to the study of morphometrical variation in the taxa involved. Discussion of homology of femoral glands in this genus might give some interesting clues for use of these glands as characters in phylogenetic analysis.

In a recent paper, published after this work was completed and submitted, PERRET (1995) described a new species, *Aubria occidentalis*, from West Africa (type-locality in Ivory Coast). Its synonymy with *Aubria subsigillata* will be discussed under this species.

MATERIAL AND METHODS

ABBREVIATIONS

The names of the collections where the studied specimens are deposited are abbreviated as follows: BMNH, British Museum (Natural History), London; CAS, California Academy of Sciences, San Francisco; K, KAZADI Mpetemba collection; KMMA, Koninglijk Museum voor Midden-Afrika, Tervuren; MHNG, Muséum d'Histoire naturelle de Genève; MNHN, Muséum national d'Histoire naturelle, Paris; MRHN, Institut Royal des Sciences naturelles de Belgique, Brussels; NMW, Naturhistorisches Museum, Wien; ZMB, Zoologisches Museum, Berlin; ZMH, Zoologisches Museum, Hamburg.

For abbreviations of measurements, see Table I.

SPECIMENS STUDIED

For this study I measured 117 adult and juvenile frogs from 41 different collecting sites. Of these, samples from 31 sites contain adult specimens (a total of 65 individuals), including 21 localities with one specimen, 5 with two, 4 with three to eight and one locality with 15 adult specimens. As my data were collected over a five-year period, in the beginning the list of measurements was not exhaustive and consequently some individuals had to be excluded from statistical analysis.

COMPOSITION OF SAMPLES

The unit used for morphometrical analysis (sample) is a group of individuals of about the same age, of the same sex and of the same locality. For this purpose three age-groups

Table I. - List of abbreviations for measurements.

Abbreviations	Descriptions of measurements
EL	Eye length
EN	Distance from front of eye to nostril
FFTF	Distance from maximum incurvation of web between fourth and fifth toe to tip of fourth toe
FL	Femoral length (from sagittal axis of body to knee)
FLL	Fore limb length (from elbow to base of outer palmar tubercle)
FOL	Foot length (from base of inner metatarsal tubercle to tip of toe)
FTL	Fourth toe length (from base of proximal subarticular tubercle)
GD	Greatest diameter of femoral gland
GLDT	Distance of femoral gland to sagittal axis of body
HAL	Hand length (from base of outer palmar tubercle to tip of finger)
HL	Head length (from back of mandible to tip of snout)
HW	Head width
IBE	Distance between backs of eyes
IFE	Distance between fronts of eyes
IMT	Length of inner metatarsal tubercle
IN	Internarial distance
ITL	Inner toe length from distal edge of inner metatarsal tubercle
MBE	Distance from back of mandible to back of eye
MFE	Distance from back of mandible to front of eye
MN	Distance from back of mandible to nostril
MTFF	Distance from distal edge of metatarsal tubercle to maximum incurvation of web between fourth and fifth toe
MTTF	Distance from distal edge of metatarsal tubercle to maximum incurvation of web between third and fourth toe
NS	Distance from nostril to tip of snout
SVL	Snout-vent length
TFL	Third finger length (from base of proximal subarticular tubercle)
TFTF	Distance from maximum incurvation of web between third and fourth toe to tip of fourth toe
TL	Tibia length
TYD	Greatest tympanum diameter
TYE	Distance from tympanum to back of eye

were recognized: larvae, with a tail; juvenile frogs, from metamorphosis to sexual maturity; and adult frogs, including females with ripe ovaries and oviducts and males with well developed testes (DUBOIS, 1976). Secondary sexual characters are absent in adult *Aubria*, so it is difficult to determine adult males based on external and internal observation. But morphometrical analysis showed an important allometric difference between subadult and adult males that allows staging of the specimens. Subadult males, when analysed using the Laurent's technique (described below), cluster in a group of their own, independent of species membership, as do females (results not shown).

The best way to identify interpopulational variation is to treat the different samples separately. Only some samples have enough specimens for such comparisons. As sample sizes are usually too small for statistical analysis, I also grouped the frogs on a morphological basis, and compared the homogeneity of the two species.

In order to optimize analysis and to find an intermediate way between analysis of single population samples and species comparisons, I grouped specimens from populations of homogeneous biogeographic areas (WÜSTER & THORPE, 1992). The areas were defined following the studies of SCHIØTZ (1967) and HAMILTON (1988) who have discussed possible biogeographic zones. The following areas were used (fig. 1): (1) Sassandra Valley, Ivory Coast; (2) Ghana; (3) Kovié, Togo, (4) Nigeria, West of Niger; (5) Nigeria, between Niger and Cross Rivers; (6) lowlands of Guinean Coast south of Cross River; (7) Sangha region at the intersect of Cameroon, Gabon and Zaire; (8) western part of Zaire; (9) eastern part of Zaire (this latter division is possibly due to a discontinuity in collecting).

MEASUREMENTS AND TRANSFORMATIONS

Measurements were taken with slide-calipers and binocular microscope. The precision varies from 0.1 % to 1 % of the measurement; for distances smaller than 3 mm, precision is only 1 % to 3 %.

Snout-vent length is given in millimetres. The other measurements are expressed as thousandths (‰) of snout-vent length:

$$RX = (X / SVL) \times 1000.$$

The position of the femoral gland on the thigh is expressed as the ratio of "femur" length (FL) to distance of gland from sagittal axis: FLGLDT = (FL / GLDT) × 1000. Webbing is given as the ratio (TFTFOL) of webbing from the tip of the fourth toe to the incurvation between the third and fourth toe (TFTF) divided by the length of the foot (FOL), or as the ratio (MTFFOL) of webbing from the distal border of the metatarsal tubercle to the incurvation between the third and fourth toe (MTFF) divided by the length of the foot (FOL).

The logarithm of the measurement was used in discriminant analysis:

$$LX = \ln X.$$

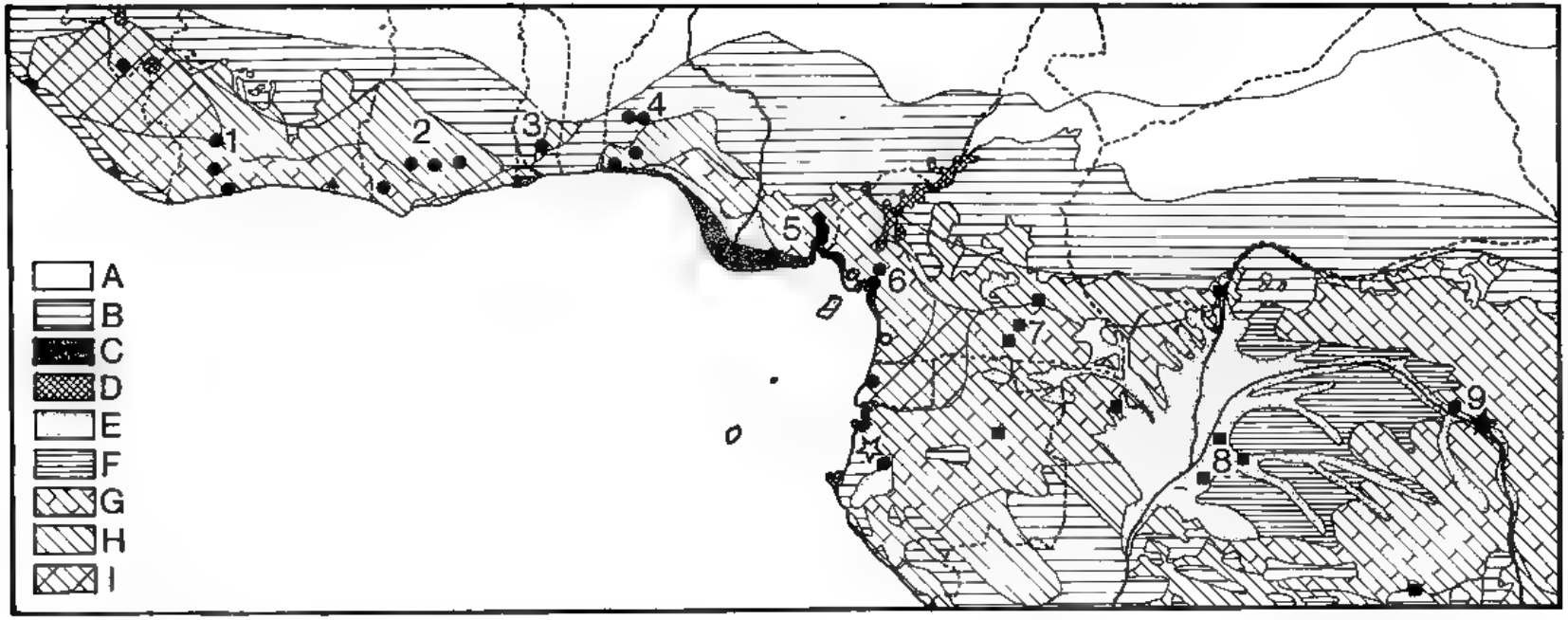

Fig. 1. – Distribution of *Aubria subsigillata* and *Aubria masako* in relation to vegetation types of tropical Africa. Black circle, *Aubria subsigillata*; white star, type-locality of *Rana subsigillata* A. Duméril, 1856, white circle, type-locality of *Phrynopsis ventrimaculata* Nieden, 1908; black triangle, type-locality of *Aubria occidentalis* Perret, 1995; black square, *Aubria masako*; black star, type-locality of *Aubria masako* Ohler & Kazadi, 1990 Forest types: A, Sudanian woodland; B, lowland rain forest; C, mangrove; D, Afromontane vegetation; E, swamp forest; F, mosaic of swamp forest and wetter lowland Guineo-Congolian rain forest; G, wetter lowland rain forest, H, drier Guineo-Congolian rain forest; I, mosaic of G and H. Biogeographical areas: 1, Ivory Coast; 2, Ghana; 3, Togo; 4, western Nigeria, 5, eastern Nigeria, 6, coastal area of Cameroon and equatorial Guinea; 7, Sangha region; 8, western Zaire basin; 9, eastern Zaire basin.

To calculate Laurent's distance, SVL was used as a size factor and all measurements were corrected as follows:

$$IX = [\ln X - \ln SVL].$$

All these corrections were used to standardize for isometric size changes.

Statistics

Mean, standard deviation, minimum and maximum were calculated for all variables of all groups on a personal computer using the SPSS program (Norusis, 1992). To estimate homogeneity, Haldane's coefficient of variation was caculated (Delaugerre & Dubois, 1985).

Non-parametric statistics permit comparison of samples of relatively small sizes (Dubois, 1984; Delaugerre & Dubois, 1985) and are therefore appropriate to use on samples from old zoological collections. Standard non-parametric statistics (Zar, 1984) were applied to compare different populations. The Kruskal-Wallis test was used on all 35 measurements to measure group homogeneity. Those measurements which showed significant differences were subsequently treated by Tukey type B tests (on ranks of variables). The Mann-Whitney U test was used for comparison between any two groups.

Laurent (1955, 1981) developed a method that uses distance measurements to compute phenograms. Cluster analysis using Laurent's procedure was made for adult males including the 35 measurements (for female frogs the sample size was too small to perform an analysis) on SPSS using Manhattan distance dissimilarities matrix and Ward's method (Norusis, 1992).

To investigate variables that distinguish the two morphologically recognized species, I performed a discriminant analysis. In the first analysis, all log-transformed measurements were included. In a further step, five variables with good discrimination characteristics (small in-group variation indicated by a relatively small Wilk's lambda) were retained to assign cases to their group based on a few measurements.

Descriptive methods

The webbing formula of Myers & Duellman (1982) was used. Tadpoles were staged following Gosner (1960). Keratodont formulae (Dubois, 1995) are given according to Annandale's (1912) system.

Results

The genus *Aubria* is widely distributed in western and central Africa (fig. 1). Its distribution includes forested areas of the following countries: Guinea, Ivory Coast, Ghana, Togo, Nigeria, Cameroon, Congo, Republic of Central Africa, Zaire, Equatorial Guinea, Gabon. In life these are very colourful frogs with a typical yellow and violet

ventral pattern (PERRET, 1966: Cameroon; PERRET, 1995: West Africa; OHLER & KAZADI, 1989: Zaire; LAURENT, personal communication, letter of 22 June 1994: Zaire). The frogs of the different regions have a superficial resemblance – they were referred to one species from the description of *Rana subsigillata* in 1856 to 1990 – but detailed morphological studies show extensive interpopulational variation.

External morphology clearly separates the frogs into two groups: one with midfemoral glands and the other with close-to-knee femoral glands (see also PERRET, 1995). In a previous study (OHLER & KAZADI, 1990), because of an insufficient number of specimens, we did not realize the presence of near-to-knee femoral glands in *Aubria masako*. The character of gland position is correlated with dorsal colour and ontogenetical variation of ventral pattern.

The morphometrical homogeneity of morphologically determined groups was tested using the Kruskal-Wallis test. The test showed no significant differences at the $P = 0.05$ level for samples of adult males and females of *Aubria subsigillata* for the following measurements and ratios: SVL, RHW, RHL, RTL, RIN, RTYD, RTFL, LFGLDT, MTFFOL. Male and female samples of *Aubria masako* were tested for the same measurements. Only the samples of males showed significant differences in the ratio RIN ($\chi^2 = 11.4$; DF = 5; P = 0.04 *).

COMPARISON OF SPECIES

Univariate comparisons

Interspecies comparisons were performed separately for males and females. The observed differences were the same as found in our previous study (OHLER & KAZADI, 1990), but treating the sexes separately underlines the differences observed. The morphometrical differences concern the head, the femoral gland position and the webbing.

Comparison of *Aubria subsigillata* and *Aubria masako* by means of Mann-Whitney U tests shows that there are statistically significant differences in both sexes in the ratios to SVL of several measurements (Table II). *Aubria subsigillata* is larger and has a longer tibia than *Aubria masako*. The webbing of its foot is more extensive. Differences exist between males and females in interspecific differentiation. In female *Aubria*, the Mann-Whitney U tests show no significant differences for several ratios concerning head (RHW, RHL, RTYD) and webbing (TFTFOL) that show significant differences in males.

Discriminant analysis

Using only five morphometric variables, adult specimens of *Aubria* can be attributed to one of the two species recognized (Table III). This permits also to classify the holotype of *Aubria occidentalis* in the *Aubria subsigillata* group. The variables are position and size of femoral gland, tibia length, metatarsal tubercle, distance between eyes and size of tympanum. They describe various aspects of the body form. Other variables were excluded because of missing values or of great variability of the measurements.

Table II. - Species differences between *Aubria subsigillata* and *Aubria masako*, all localities pooled, males and females treated separately. N, number; SD, standard deviation; V, coefficient of variation; U, Mann-Whitney U; P, probability; SUB, *A. subsigillata*; MAS, *A. masako*, for other abbreviations, see Table I and text.

Measurement	Species	N	Mean	SD	V	U	P
Males							
SVL	SUB	17	79.1	4.1	5.31	16.0	0.000***
	MAS	12	71.4	4.5	6.43		
RHW	SUB	17	349	11 63	3.37	40.0	0.006**
	MAS	12	363	16.3	4.58		
RHL	SUB	17	412	11 9	2.93	50.0	0.021*
	MAS	12	428	20.7	4.94		
RTL	SUB	17	388	14.1	3.68	30.0	0.001**
	MAS	12	368	16.0	4.26		
RIN	SUB	17	66	3.8	5.84	8.0	0.000***
	MAS	11	60	1.7	2.89		
RTYD	SUB	17	73	5.9	8.20	33.0	0.002**
	MAS	12	83	8 2	10.09		
LFGLDT	SUB	13	370	45.7	12.59	0.0	0.001***
	MAS	6	516	21.3	4.30		
RMTTF	SUB	15	242	15.3	6.42	21.0	0.006**
	MAS	9	222	14.7	6.80		
TFTFOL	SUB	17	445	38.8	8.85	42.0	0.008**
	MAS	12	480	27.0	5.75		
Females							
SVL	SUB	17	86.0	4.1	4.84	28.0	0.004**
	MAS	10	78.4	6.0	7.84		
RHW	SUB	17	348	9.3	2.71	77.0	0.688 ns
	MAS	10	344	21.0	6 26		
RHL	SUB	17	403	13.9	3.50	68.0	0.393 ns
	MAS	10	399	16.1	4.14		
RTL	SUB	17	385	17.4	4.59	6.0	0.000***
	MAS	8	352	18.1	5.30		
RIN	SUB	16	66	4.3	6.62	13.0	0.001***
	MAS	9	58	3.6	6.38		
RTYD	SUB	17	74	5.7	7.81	47.0	0.056 ns
	MAS	10	79	7.0	9.08		
LFGLDT	SUB	13	334	55.8	17.03	0.0	0.000***
	MAS	8	487	53.4	11.31		
RMTTF	SUB	15	230	18.8	8.31	3.0	0.003**
	MAS	5	196	9.72	5.21		
TFTFOL	SUB	16	450	37.5	8.46	61.0	0.317 ns
	MAS	10	456	28 0	6.29		

Table III.- Use of discriminant function to distinguish *Aubria subsigillata* and *Aubria masako* (adult males and females together) based on 5 morphometric parameters (see "Material and methods").

A. Statistical significance

Eigenvalue	Canonical correlation	Wilks Lambda	Chi-square	Degrees of freedom	P
3.8584	0.8912	0.2058	68.76	5	0.0000

B. Standardized canonical discriminant function coefficients.

Morphometric character	Function 1
Tibia length	0.69064
Tympanum diameter	- 0.34850
Distance of femoral gland	- 0.45144
Diameter of femoral gland	- 0.38166
Distance between posterior part of eyes	0.60099

C. Classification success

Actual group	Predicted group	
	Aubria subsigillata	*Aubria masako*
Aubria subsigillata	30 (100%)	0
Aubria masako	0	15 (100%)
Aubria occidentalis holotype (ungrouped)	1	0

Laurent's morphometric distance

Measurements of males from all localities were included in LAURENT's (1955, 1981) procedure, which separated the two species very clearly (fig. 2). One group includes the type-specimens of *R. subsigillata* and *A. occidentalis* and corresponds to the morphologically determined *A. subsigillata*. The second group includes specimens from Masako, Boteke and Sangha and the type series of *A. masako*.

COMPARISON OF POPULATIONS

The inter-group differences that are significant at the $P < 0.05$ level obtained by the Tukey test are shown in fig. 3 for males and in fig. 4 for females.

In males, the differences between OTUs 2, 3, 4, 6 and OTUs 7, 8, 9 appear clearly. In females, where the number of specimens is very small even when the populations are grouped, the relations between the OTUs appear slightly different. The population 3 of *A. subsigillata* from Togo seems to be morphologically closest to *A. masako* populations 7, 8, 9. Comparison of the specimens from population 7 (Sangha region) with populations from western Zaire (8) and eastern Zaire (9) shows that they should be included in *A. masako*.

DETAILED ACCOUNT OF SPECIES

Aubria Boulenger, 1917

Aubria Boulenger, 1917: 988. – Type species by monotypy: *Rana subsigillata* Duméril, 1856

The types of *Aubria subsigillata* and of *Aubria masako* have been described in detail (DUMÉRIL, 1861: 224, pl. XVIII fig. 1, OHLER & KAZADI, 1990). Here I will give a description of the variation and of sexual dimorphism within populations, descriptions of tadpoles and young frogs, and diagnoses for the two species.

Aubria subsigillata (A. Duméril, 1856)

Rana subsigillata A. Duméril, 1856: 560. – Holotype: MNHN 1566. Type-locality: Gabon.
[*Rana (Aubria) subsigillata*]: BOULENGER, 1917: 988.
Aubria subsigillata: LAURENT, 1953: 27.
Phrynopsis ventrimaculata Nieden, 1908: 499 – Holotype: ZMB 20134. – Type-locality: Longji, Cameroon. – Synonymy fide OHLER & KAZADI, 1990.
Aubria occidentalis Perret, 1995: 258 – Holotype: MHNG 2129.17. – Type-locality: Banco forest reserve, Ivory Coast. – New synonymy.

Specimens studied. – GABON. MNHN 1566, holotype of *Rana subsigillata* A. Duméril, 1856; Biligone River: MNHN 1974.1130; 50 km SW of Lambaréné: MNHN 1901.564. – EQUATORIAL GUINEA. Benito River: ZMH A.03133. – CAMEROON. Yabassi District (4°N, 10°E): BMNH 1938.6.10.9; SW-Province, "Korup": BMNH 1982.746; Yaoundé Road, 4

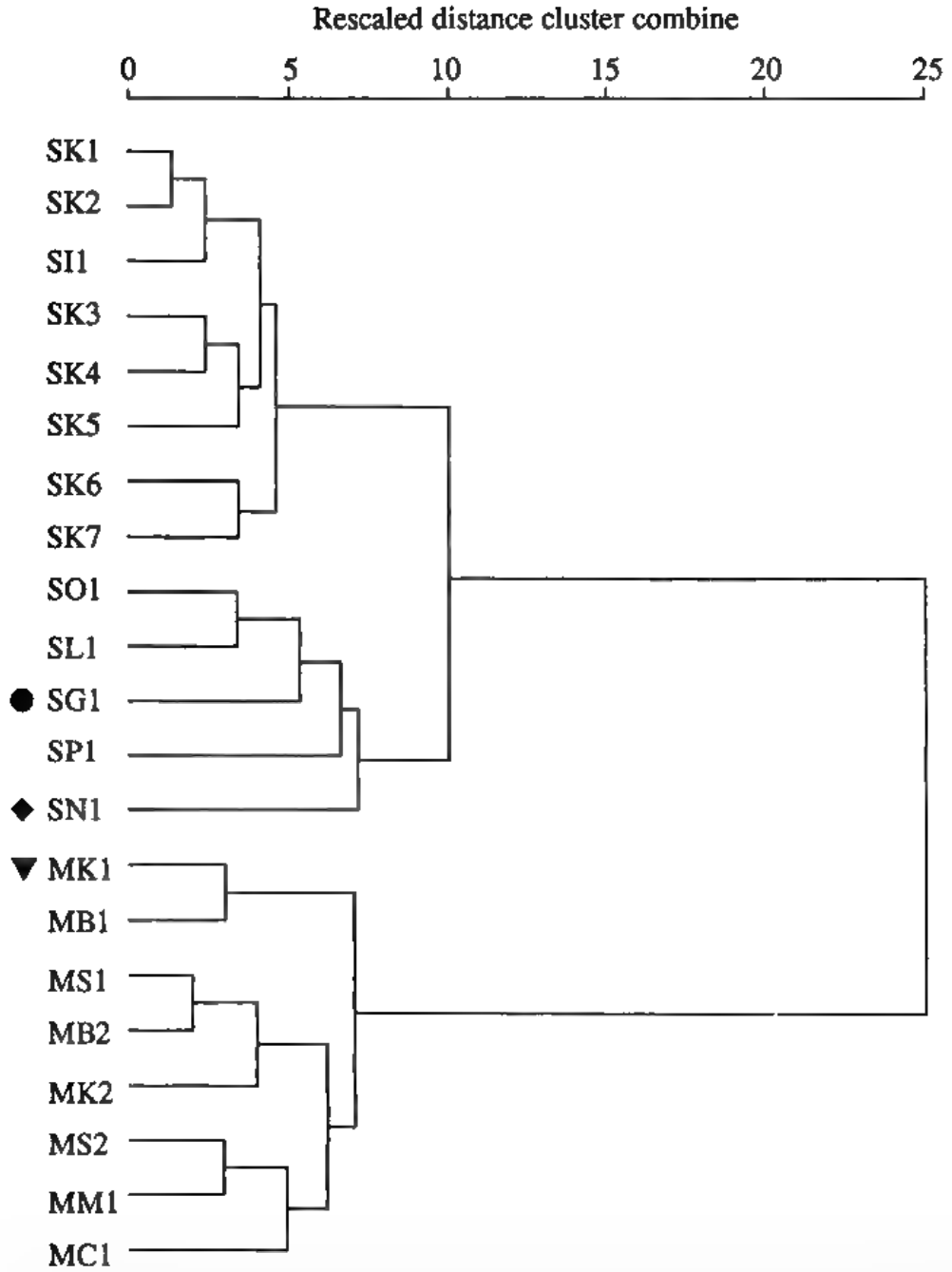

Fig. 2. – Phenogram of 21 adult males belonging to the species *Aubria subsigillata* and *Aubria masako*: Laurent's distances based on 35 measurements were included in distance calculations, using Ward's method. S, *A. subsigillata*: K, Kovié, Togo: 1, MNHN 1989.2054; 2, MNHN 1993.1462; 3, MNHN 1989.2056; 4, MNHN 1993.1966, 5, MNHN 1993 1469; 6, MNHN 1989.2050; 7, MNHN 1989.2053; I, Ibadan Swamp, Nigeria: 1, BMNH 1964.237; L, Lambarene, Gabon: 1, MNHN 1901.564; O, Obuasi, Ghana: 1, BMHN 1917.4.13.13; G, Gabon: 1, MNHN 1566, holotype of *R. subsigillata* (black cercle), P, Port Harcourt, Nigeria: 1, BMNH 1956.1.10.84; N, Banco forest, Ivory Coast: 1, MHNG 2129.17, holotype of *A occidentalis* (black square). M, *A. masako*: K, Kisangani, Zaire: 1, MNHN 1989.2775, holotype of *A. masako* (black triangle); 2, MNHN 1989.3305, paratype; B, Boteke, Zaire. 1, KMMA 85.30.B.368; 2, KMMA 85.30.B.362; S, Sangha, Congo: 1, MNHN 1993.2831; 2, MNHN 1989.2830, M, Mabali, Zaire: 1, CAS 145297; C, Coquilhatville, Zaire: 1, CAS 113967.

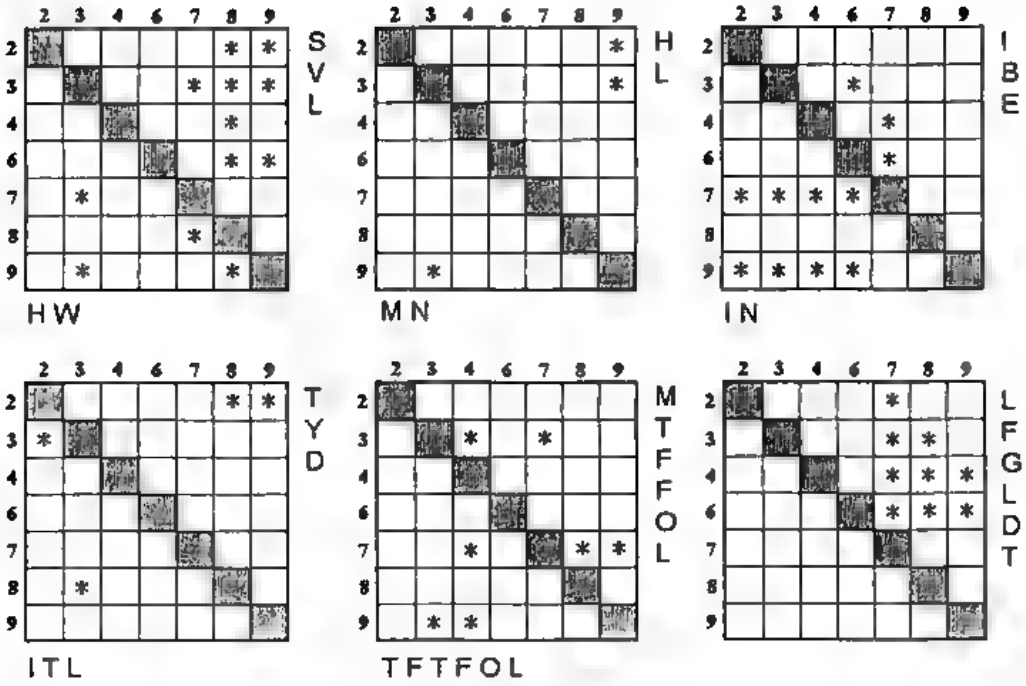

Fig. 3. – Significant differences ($P < 0.05$) of measurements among males of *Aubria*, grouped populations of biogeographically homogeneous areas, using the Mann-Whitney U test. 2, Ghana; 3, Togo; 4, western Nigeria; 6, coastal area of Cameroon and equatorial Guinea; 7, Sangha region; 8, western Zaire basin, 9, eastern Zaire basin. For key to measurement abbreviations see Table I.

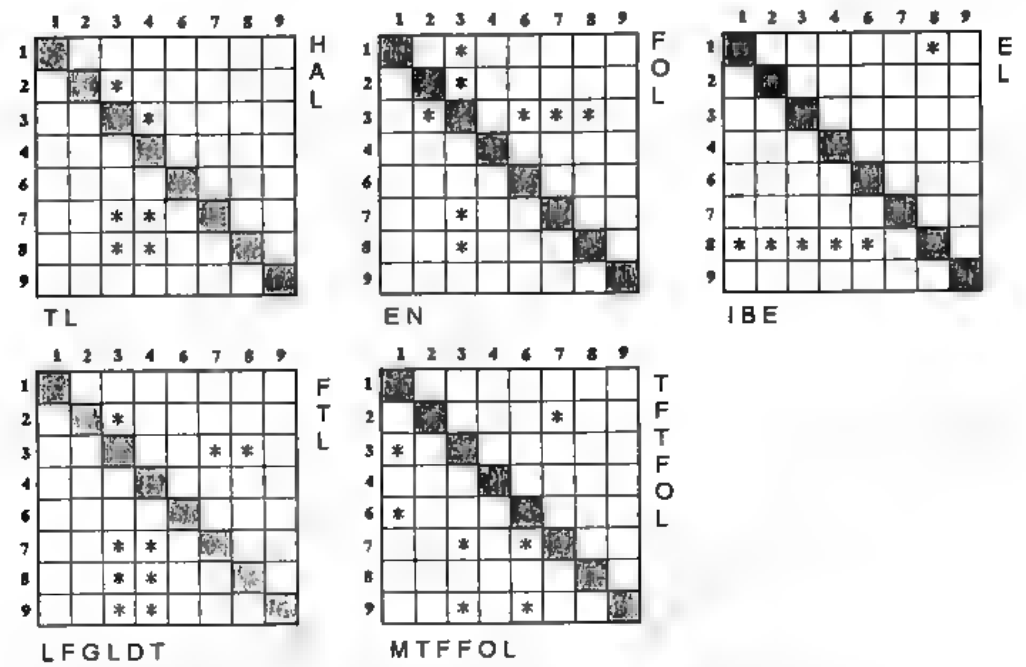

Fig. 4. Significant differences ($P < 0.05$) of measurements among females of *Aubria*, grouped populations of biogeographically homogeneous areas, using Mann-Whitney U test. 1, Ivory Coast; 2, Ghana; 3, Togo; 4, western Nigeria; 6, coastal area of Cameroon and equatorial Guinea; 7, Sangha region, 8, western Zaire basin, 9, eastern Zaire basin. For key to measurement abbreviations see Table I.

mi E of Douala: CAS 103804. – NIGERIA. NMW 2552; Calabar, Edge of Great Kwa River: BMNH 1980.1275; Calabar, 20 km north on MCC Road: BMNH 1980.1276; Calabar, 15 km north on MCC Road: BMNH 1980.1277; Ibadan: BMNH 1969 3000; Ibadan Swamp: BMNH 1964.237; Ijebu Ode: BMNH 1969.2999; Lagos: NMW 2555; Port Harcourt: BMNH 1956.1.10.84. – GHANA. Eastern Region, Tafo Cocoa Research Institute: CAS 146050-51, CAS 144214-215; Eastern Region, Kadé, Agricultural Research Station of University of Ghana: CAS 103783, CAS 103818, CAS 103836, CAS 104016, CAS 104051, CAS 125534; South Ashantee, Obuasi: BMNH 1917.4.13.12-13; Western Region, "Wasaw Akropong" (north of Tarkwa): CAS 97967. – TOGO. Kovié: MNHN 1989.2047-2056 and 1993.1463-1472; Mission Tové: MNHN 1989.4094. – GUINEA. Diéké: MNHN 1920.147. – IVORY COAST. Daloi-Lobo: MNHN 1993.929-930 (formerly A 929 and A.930); Sassandra: MNHN 1993.2832-2834; Soubré: MNHN 1989.4095; Banco forest reserve: MHNG 2129.17, holotype of *Aubria occidentalis* Perret, 1995.

Diagnostic characters. – *Aubria subsigillata* has femoral glands in a position midway from knee to vent in a ventral position on the thigh (fig. 5). The femoral gland is rounded. Feet are more webbed (I 1 – 2 II 1 – 2 III 1 – 2.5 IV 2.5 – 1 V) than in *Aubria masako*. Coloration of dorsum is uniform and structure of skin is smooth. Ventral mottled pattern tends to disappear in adult frogs on throat first.

Synonymy. - Direct comparison of the holotype of *Aubria occidentalis* Perret, 1995 with the holotype of *Rana subsigillata* A. Duméril, 1856 (see DUMÉRIL, 1861, pl. XVIII, fig. 1) and morphometric analysis (fig. 2) clearly indicates synonymy of the two names. This study includes also material from Ghana, included in the list of localities of paratypes of *A. occidentalis* by PERRET (1995). Another piece of evidence is given by the distribution of *Aubria occidentalis*, which coincides exactly with the distribution of *Aubria subsigillata*. PERRET (1995) did not examine material of both species from Gabon (see OHLER & KAZADI, 1990: 34, fig. 6, for the "mid-thigh gland" form of Gabon). In particular, he did not study the type specimen of *Rana subsigillata*. Morphological analysis does not show major differentiation within frogs of the genus *Aubria* from western Africa. Morphometrical comparison of larger samples of *Aubria subsigillata* from Gabon with western populations and studies using other techniques (such as bioacoustics), might lead later to division of *A. subsigillata*. The name *Aubria occidentalis* Perret, 1995 would then be available for the western taxon.

Sexual dimorphism. – In the genus *Aubria*, no secondary sexual characters are known. Males lack vocal sacs, nuptial pads on fingers, and spinosities on different parts of the body. Thus sexes cannot be distinguished externally.

A sample (from Kovié, Togo) large enough to allow statistic comparison between males and females was studied. The most striking morphometrical difference between males and females is their size (Table IV). Females are significantly larger than males. Of the ratios to SVL of the 34 other measurements taken, only two show significant differences (Table IV). The eye is relatively longer in males than in females and the femoral gland is nearer to the base of the thigh (sagittal axis) in females than in males.

Fig. 5. – Position of femoral glands in females of *Aubria subsigillata* (above, MNHN 1993.1468, Kovié, Togo) and of *Aubria masako* (below, MNHN 1993.2830, Ivindo, Gabon). Ventral view of right thigh. Scale: 10 mm.

Morphological comparison shows that femoral glands are more developed in females than in males. These composite glands are structures that are prominent and more brightly colored and yellowish in females (fig. 6). In males the presence and position of the glands can be recognized primarily by colour.

Table IV. - Morphometrical differences between males and females of a population of *Aubria subsigillata* from Kovié, Togo. SD, standard deviation; MIN, minimum; MAX, maximum; U, Mann-Whitney U; P, probability.

Measurement		Mean	SD	MIN	MAX	U test
SVL	♂ n=8	77.83	4.70	67.2	83.4	U=0
	♀ n=7	86.44	2.60	83.6	89.7	P<0.001 ***
REL	♂ n=8	108.73	6.09	100.7	121.0	U=7.0
	♀ n=7	100.74	4.94	93.7	104.8	0.01<P<0.05 *
RGLDT	♂ n=8	159.70	13.64	140.1	183.0	U=7.0
	♀ n=7	130.55	22.33	95.3	164.6	0.01<P<0.05 *

Table V. - Measurements (in mm) on tadpoles of *Aubria masako* as compared to *Aubria subsigillata* from SCHIØTZ (1964). SUB, *Aubria subsigillata*; MAS, *Aubria masako*.

	SUB	MAS (stage 37)	MAS (stage 40)
Total length	39	41.3	46.4
Snout-vent	16	18.8	19.0
Eye-eye	2.2	3.24	3.44
Nare-nare	2.0	1.55	1.75
Tail height	7.5	7.78	8.80
Keratodont formula	2:4+4/3	2:3+3/3	2:4+4/3

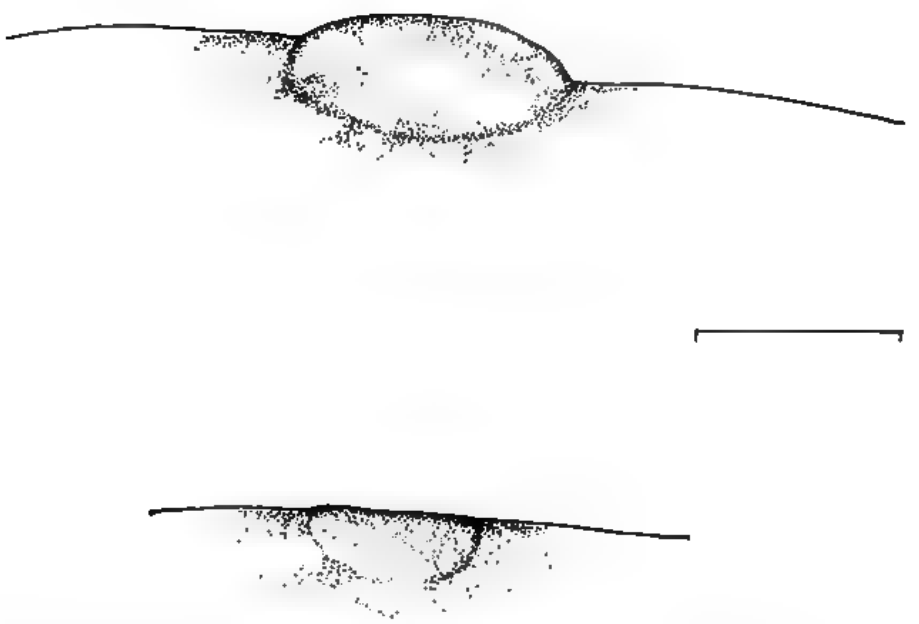

Fig. 6 – Sexual differentiation of femoral glands in *Aubria subsigillata* from Kovié, Togo. Above gland of left thigh of female (MNHN 1993.1468) and below gland of left thigh of male (MNHN 1993.1466). Scale: 5 mm

Larval characters. – A larva of this species was described by SCHIØTZ (1963) who gave drawings of a general view and of mouthparts. Larval keratodont rows formula: 2:4+4/3 (Table V). Body length: 16 mm; tail length: 23 mm.

Distribution. – The species occurs in western and central Africa to Gabon in the coastal area. The westernmost area of *Aubria subsigillata* is the valley of Sassandra river in Ivory Coast and Diéké in southern Guinea, near the Liberian border (fig. 1). Lambarene (Gabon, 0°41'S) is the most southern collecting point known.

Aubria masako Ohler & Kazadi, 1990

Aubria masako Ohler & Kazadi, 1990: 29. – Holotype: MNHN 1989.2775. – Type-locality: Masako forest, near Batibongena village, 15 km from centre of Kisangani on the ancient road to Buta, Zaire.

Specimens studied. – ZAIRE. Masako: MNHN 1989.2775, holotype; MNHN 1989.3305-3311, K 1049, K 1266, K 1324-1326, K 1463-1464, K 2505, K 2510-2511, K 2520, K 2528, K 2626, K 3500, K 3933; Boteke swamp: KMMA 85.21.B.123-141, KMMA 85.30.B.365-367; near Coquilhatville: CAS 113967-113968; Equateur Province, Bikoro Territory, Lotende Swamps near Mabali: CAS 145297; Sankuru Province, Lodja Territory, Omaniundu: CAS 145276; Yangambi: KMNH 15478. – GABON. Ivindo: MNHN

1993.2830-2831. – CONGO. Sangha: MNHN 1945.13, 1994.1665-1666. – REPUBLIC OF CENTRAL AFRICA. Zimba: MNHN 1993.4451. – CAMEROON. ZMH A.03134; Batouri District (4°N 14°25'E): BMNH 1934.12.1.2; Batouri District (3°75'N 13°75'E): BMNH 1937.1.1.1; Bitye: NMW 2554 (3 specimens), NMW 2557; Ya River (Dja): NMW 2553; Efa Yong (Efangono): NMW 2556.

Diagnostic characters. – The femoral glands are not in mid-femoral position, but closer to the knee (fig. 5) and in a more posterior position than in *Aubria subsigillata*. The femoral glands are more elongated and feet are less webbed (I 1½ – 2 II 1 – 2½ III 2 – 3 IV 2½ – 1 V) than in *Aubria subsigillata*. The dorsal pattern is clear with darker spots on slight warts. A mid-dorsal line may be present. In adults the ventral mottled pattern disappears on vent region, and remains more visible on throat.

Chresonymy. – Specimens from Ivindo forest (Gabon) listed under the name *Aubria subsigillata* by PERRET (1995) are morphologically similar to specimens from Zaire and should bear the name *Aubria masako*.

Adult morphology. – Before this study, this species was known only from the type-locality and neighbouring regions (OHLER & KAZADI, 1990). Morphological variation in this group is very important. In fact three specimens of the Sangha region of Cameroon, that could not be assigned clearly to one of the two species in 1990 due to fixation problems, are here tentatively placed in this group on the basis of femoral gland position, as well as other specimens from other localities, although some of them show major differences with the specimens from the type-locality. In the population from western Zaire (Boteke), that is different in many morphometrical characters (fig. 4), 4 specimens out of 20 (20 %) have a mid-dorsal stripe, a frequency lower than that observed in the type-locality (i.e. 65.2 %, N = 23) (OHLER & KAZADI, 1990).

Size at metamorphosis. – I was able to study a sample from Boteke (Zaire) composed of specimens of all three age-groups. This series includes numerous froglets in metamorphosis, exhibiting the specific characters already present, and two tadpoles with complete mouthparts.

The size just before metamorphosis (Gosner stage 45) varies from 18.3 to 20.0 mm (mean SVL = 19.37; N = 13; SD = 0.46).

After metamorphosis (mean SVL = 22.6 mm; extremes 22.1-23.2 mm; N = 3; SD = 0.55), the froglets already have femoral glands in the characteristic position. These three specimens are without mid-dorsal line and ventral pattern is complete (dark with whitish spots).

Larval characters. – Two tadpoles with complete mouthparts were studied (Table V; fig. 7). The younger tadpole (Gosner stage 37) is lighter in colour than the older tadpole (Gosner stage 41) and metamorphosing tadpoles. The vent and lateral parts of the body both already have the typical coloration of *Aubria*: dark with white spots. In general appearance it resembles the tadpole figured by SCHIØTZ (1963). Measurements are very similar with the exception of internarial distance that seems to be smaller in *masako*

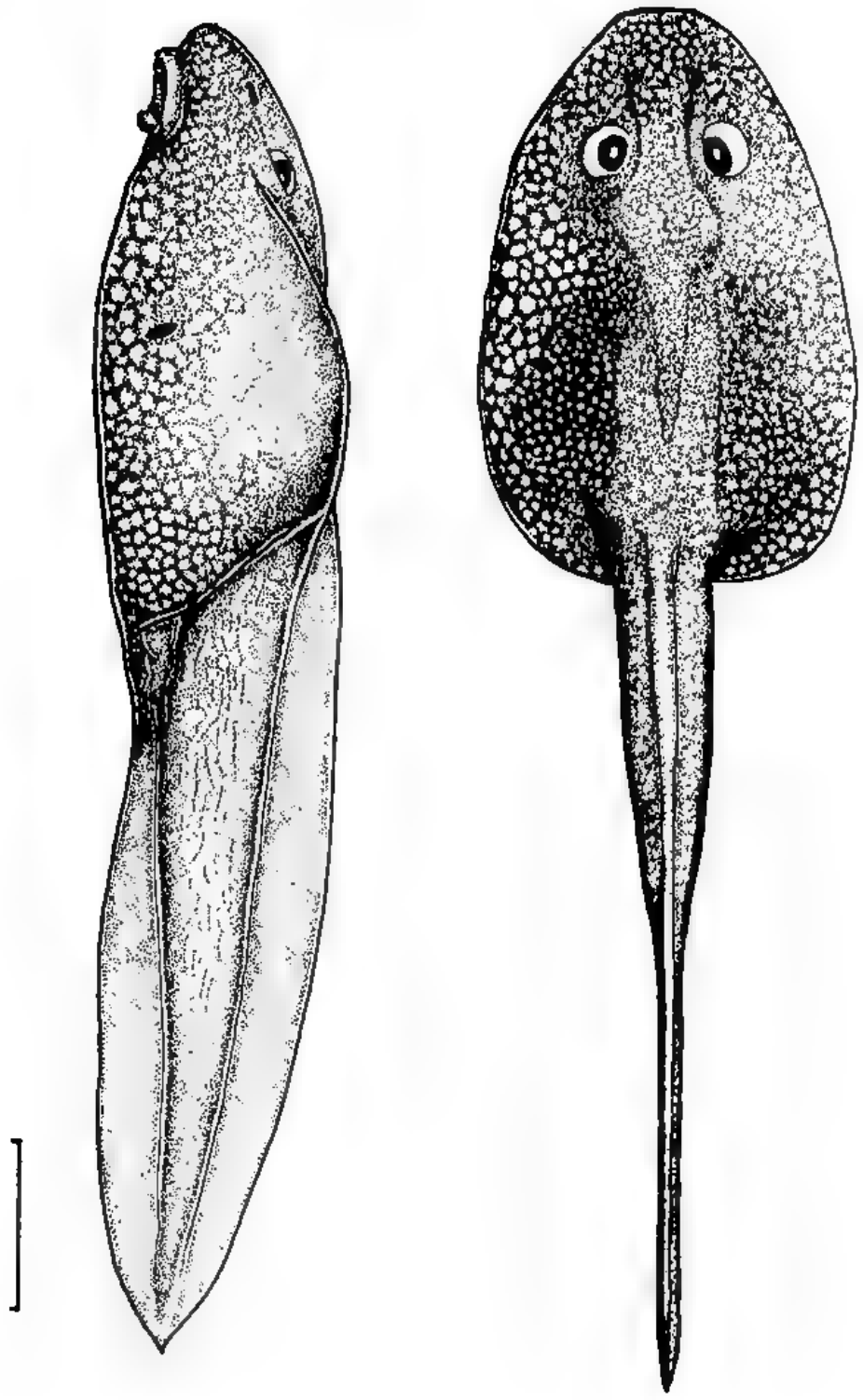

Fig. 7. – Tadpole of *Aubria masako* (KMMA 85 21 B.141) from Boteko, Zaire (dorsal and lateral view, Gosner stage 37). Scale: 5 mm.

tadpole (51 ‰ of total length in the single tadpole of *subsigillata* and 38 ‰ in both tadpoles of *masako*) just as it is in adults (Table II). All characters, including the keratodont rows, must be studied on larger samples of tadpoles of both species.

DISCUSSION

SPECIES DIFFERENTIATION IN THE GENUS *AUBRIA*

Geographic morphological variation seems to be less important in *Aubria subsigillata* than in *Aubria masako*. In *A. subsigillata* morphology seems to be very similar over the wide range of distribution from Ivory Coast to Gabon despite important collection gaps. No major morphometrical differences were detected between samples of different geographic origins.

It seems doubtful that gene flow alone can explain this homogeneity Some populations must have been separated for an extended period of time, however no major variations in morphology can be observed that would indicate heterogeneity in the underlying genotype. Such observation lends support to the hypothesis of the unity of the genotype (MAYR, 1975) due to molecular, genetic and evolutionary constraints.

In contrast, *A. masako* seems to be a more variable group with probably several subgroups, at least one in western central Africa (Gabon, Congo, western Zaire), and another one in eastern and central Zaire. These two subgroups might be interpreted as subspecies, but more detailed data on distribution and morphological variation of *Aubria masako* should clarify the situation.

PHYLOGENETIC CONSIDERATIONS

At present there is no general accepted classification of ranid frogs (DUELLMAN, 1975; FROST, 1985; LAURENT, 1986; FEI et al., 1990; DUBOIS, 1992; BLOMMERS-SCHLÖSSER, 1993; EMERSON & BERRIGAN, 1993), as the classification of Ranoidea is in a state of revolution. It did not change for almost a century. BOULENGER (1920) proposed to subdivide the genus *Rana* in several subgenera. This was followed in the African region by subsequent recognition of several genera. But the same did not occur in the other areas, particulary Asia, where members of this group show the most important radiation. Works on phylogeny and systematics of Asian ranids were published in Chinese (LU & HU, 1961; FEI et al., 1990) and ignored in Western countries. The work of CLARKE (1981) was a first step to analyse relationships of ranid subgroups and led DUBOIS to propose his 1987*b* classification. DUBOIS (1992) included many new data on morphology of ranid frogs. As the main problem remains the classification of the genus *Rana*, which is clearly polyphyletic, subdivision of this genus in provisional subgenera allows formulation of phylogenetic hypotheses more easily. Further work is needed to resolve the higher categories (tribes becoming subfamilies, subfamilies becoming families, etc.). This does not affect nomenclatural stability, because generic allocation of many species in *Rana* is

maintained. Nevertheless keeping of Dicroglossinae in the genus *Rana* is no longer possible, as members of the Dicroglossinae comprise a well identified monophyletic group (OHLER & DUBOIS, 1989; EMERSON & BERRIGAN, 1993). For these reasons, I here follow DUBOIS' (1992) classification. According to this classification, the genera *Aubria* and *Pyxicephalus*, which have long been known to be closely related (PROCTER, 1919), constitute together the subfamily Pyxicephalinae.

Femoral glands are present in phylogenetically distinct anuran groups. They can be found in Pelobatidae and in several families of Ranoidea. In the Mantellidae, Phrynobatrachidae and the ranid subfamily Raninalinae, the glands are present in male specimens only or are much more conspicuous in males than in females. This suggests that these glands are not homologous to those present in the Pyxicephalinae, where the reverse is observed (see below). In fact such a puzzling distribution of this structure among anurans suggests a high level of homoplasy. For Pyxicephalinae the presence of femoral glands can be interpreted as an apomorphic character supporting the monophyly of the group.

Three important characters allow distinction of the two species of *Aubria* (see also PERRET, 1995): the position of the femoral gland, the mid-dorsal line and the ventral pattern. Only the position of the femoral glands is constant in all post-metamorphic specimens of a given species. In some old alcohol preserved specimens that were dried and had their colour changed to blackish, the femoral glands are hard to recognize.

The position of the glands is either mid-femoral or close-to-knee. No ontogenetic changes were observed. In specimens (males and females) of the genus *Pyxicephalus*, femoral glands are found in the close-to-knee position (FLGLDT = 534; SD = 84.5; N = 23) as in *A. masako*. No variation in gland position has been found in this genus (SUEUR & OHLER, in preparation). This position of femoral glands is therefore interpreted here as the plesiomorphic character state. The mid-thigh position of femoral glands present in *A. subsigillata* is here interpreted as the apomorphic character state.

The mid-dorsal line is a feature observed in two of the 40 populations studied; both have femoral glands in close-to-knee position. In other frog species, the presence-absence of a mid-dorsal line has been shown to be determined by a single gene (MORIWAKI, 1953; DUBOIS, 1979; BERGER & SMIELOWSKI, 1982). This coloration pattern can be found in *Pyxicephalus* and in the Dicroglossinae, the possible sister-group of the Pyxicephalinae (CLARKE, 1981, DUBOIS, 1987*b*, 1992). In the populations of *A. subsigillata*, a mid-dorsal line was never observed. The permanent absence of the mid-dorsal line, here viewed as a secondary loss of an allele from the gene pool of the species, is the apomorphic character state. The possible presence of mid-dorsal line is the plesiomorphic state of this character.

The third character is the ventral colour pattern Three different states can be distinguished and form an ordinated transformation ($0 \rightarrow 1 \rightarrow 2$). The absence of ventral coloration pattern can be found in Dicroglossinae and in *Pyxicephalus* and is the plesiomorphic state (0). The intermediate state is the presence of a ventral pattern of whitish rounded patches on a dark ground in young ontogenetic stages (older larvae and juvenile frogs) and the gradually disappearing in adult stage (1). This is observed in *Aubria masako*. In the third state, the colour pattern remains distinct in adults (2). I interpret this as a partial paedomorphism sensu DUBOIS (1987*a*): a somatic feature (here, ventral colour pattern) shows juvenile character in an adult phenotype. This state is present in *Aubria subsigillata*.

The subfamily Dicroglossinae, represented by the genera *Hoplobatrachus* (species *occipitalis* and *tigerinus*) and *Conraua*, is defined by CLARKE (1981) by one character: 7,1 P − anteriorly reduced preorbital process of pars fascialis of the maxilla.

The Pyxicephalinae (*Pyxicephalus* and *Aubria*) are a monophyletic group defined by CLARKE (1981) by 5 apomorphic characters: 2,1 − presence of cranial exostosis, 4,1 P − presence of occipital canal; 6,1 − a zygomatic ramus of the squamosal much longer than the otic ramus and articulating with the postorbital process of the pars fascialis of the maxilla; 14,3 − strong overlap of the anterior border of parasphenoid ala by the medial ramus of the pterygoid articulating along at least 1/2 the anterior width of the parasphenoid ala; 19,1 − sternal style a long bony element tapering markedly anteriorly to posteriorly.

The genus *Pyxicephalus* is defined by three apomorphic characters: 9,1 − pterygoid process of maxilla well developed, directed postero-medially, overlapping anterior ramus of pterygoid, with which it forms a suture, 16,1 P − base of the omosternum slightly forked, the greatest space between the arms being less than half the width of one arm; 22,4 − terminal phalanges of fingers and toes reduced, almost cone-like.

Superposition of the new characters defined above on the cladogram proposed by CLARKE (1981) (fig. 8) corroborates the phylogeny of CLARKE (1981), and one apomorphic character of *Aubria* has been defined.

BIOGEOGRAPHY

Aubria is generally considered as a genus limited to high forests (LAMOTTE, 1966, SCHIØTZ, 1967). Comparing our locality data with vegetation maps (WHITE, 1983) seems to agree with these statements. Nevertheless, one must be very careful with this kind of data, because geographical maps may not reflect the detailed local situation. In many regions, forest is very sparse and fragmented due to human activities. When precise collection data are available for *Aubria*, they mention forest localities or forest border areas. Distribution of the two species is in fact closely related to rain forest distribution in Africa

African tropical forest is not a homogeneous ecosystem. Several forest types can be recognized (WHITE, 1983). Several biogeographic refuges or core areas can be recognized from distribution data of vertebrates (HAMILTON, 1988). Two major refugia areas can be found on the western and eastern border of the Zaire basin. Two biologically somewhat more impoverished areas can be found in western Africa in Sierra Leone and Liberia, and in eastern Ivory coast and western Ghana. The Zaire basin seems to be an area of disjunct distribution for many taxa. The distribution gap corresponds to the area of the swamp forest (WHITE, 1983). *Aubria* seems to be absent from this swamp forest; this could be due to the presence in this forest of an open canopy which resembles secondary forest, where *Aubria* seems to be absent (SCHIØTZ, 1967).

However, AMIET (1989) included *Aubria* among the species occurring in modified forest habitats. He found choruses in secondary habitats, in forest swamp areas or even

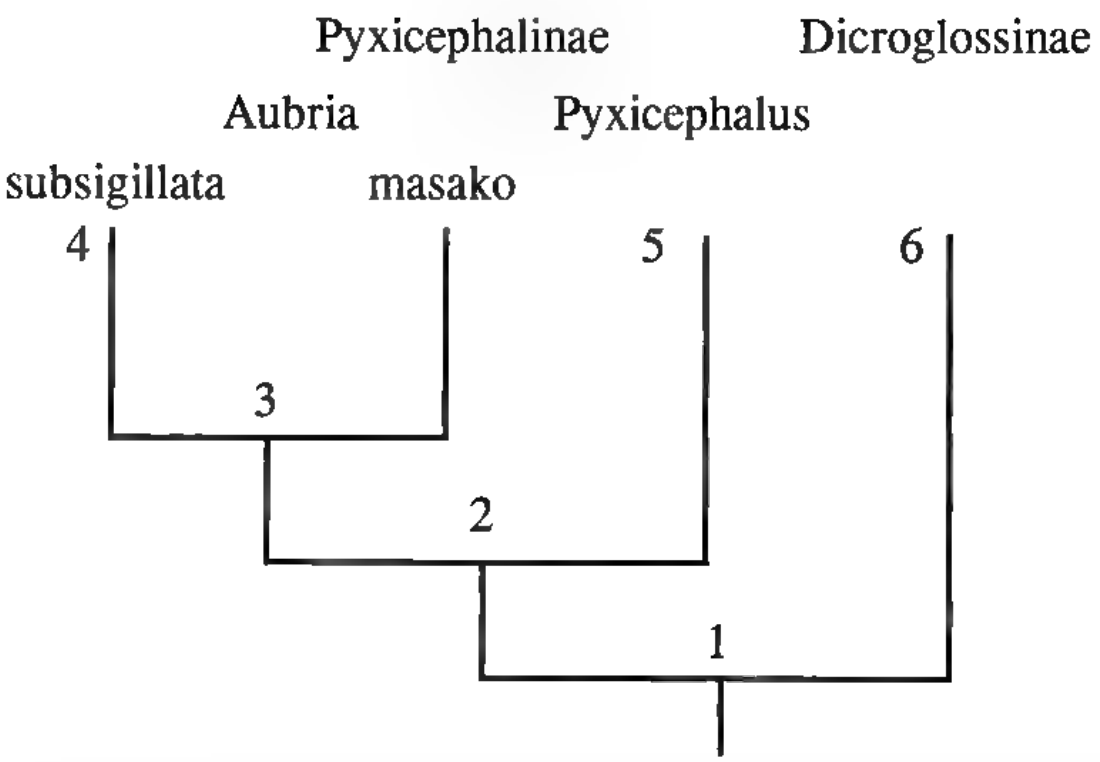

Fig. 8. – Interrelationships of Pyxicephalinae as proposed by CLARKE (1981) The tree was rooted with Dicroglossinae (genus *Hoplobatrachus*) as the outgroup. Nodes 1, 2 (in part), 5 and 6 have been defined by CLARKE Node 2 is corroborated by one additional synapomorphy discussed in the present study (femoral glands present in both sexes close to knee position). Nodes 3 and 4 are each supported by an apomorphic state of the transformation series of the ventral pattern coloration (node 3: ventral colour pattern in juvenile stages; node 4: ventral colour pattern also in adult stage). Node 4 is further supported by an additional apomorphy (permanent absence of mid-dorsal line).

in open swamp areas (AMIET, personal communication). Further exploration of central African forest should clarify the ecological preferences of the two species of *Aubria.*

Two species of *Aubria* are present in the central African forest area. From geographic distribution, *Aubria subsigillata* appears to be a form distributed in lowland rain forest near the west and central African coast. *Aubria masako* is found in the basin of the Congo river and in the Cameroonian plateaus In Cameroon and Gabon, where the two species occur, the distinction of a coastal form (*subsigillata*) and a "continental" form (*masako*) is clear (PERRET, 1995, as *occidentalis* and *subsigillata* respectively).

The character analysis indicates an eastern Zaire basin origin for *Aubria.* Starting from the Zaire basin, *Aubria* has colonized the entire area of tropical forest of central and western Africa. Splitting up of the forest in historical dry periods might be a factor of the evolution of two species. The observed intraspecific variation might result from ecological isolation. Further investigations using call analysis and adequate samples for morphometrical studies might discriminate further taxa.

FUNCTION OF FEMORAL GLANDS

The femoral glands, either in mid-femoral or close-to-knee position, are always present, in males, females and juveniles examined (smallest specimen in metamorphosis, KMMA 85.21.B.125: SVL = 18.3 mm). Their observation is sometimes difficult due to fixation problems. Often a small incision of the skin in the presumed gland region can resolve the doubt: if a gland is present, the skin shows a basal thickening due to the large size of glandular cells.

The difference in position of the femoral glands is distinctive but not sufficiently significant to question gland homology. The correlation of a large set of characters between the two species and to their outgroup *Pyxicephalus* confirms this homology.

Nothing is known of the biological signification of this gland. In a large sample of *Aubria subsigillata* (23 specimens from Kovié, Togo), the development of the gland (prominence) seems to depend on the sex of the frogs. Adult females have clearly more prominent glands than males whose glands can be recognized only by their different colour (fig. 6). This is confirmed by PERRET's (1995) observation on samples from Ivory Coast (under the name *A. occidentalis*). Nevertheless in other specimens it is still difficult to distinguish male and female specimens on external characters only. In further investigations attention should be paid to seasonal variation of femoral gland size, development and colour in *Aubria*.

In Mantellidae and Phrynobatrachidae, femoral gland aggregates are visible or more developed in males. As femoral glands, and other ventrally positioned glands, of males are in contact with females during amplexus, a stimulatory function of femoral gland secretions is assumed (DUELLMAN & TRUEB, 1985: 58).

In *Aubria*, schooling of tadpoles has been observed (SCHIØTZ, 1963). A similar behaviour is known in *Pyxicephalus adspersus*, another species of the subfamily Pyxicephalinae (POYNTON, 1964: 95). Tadpoles are "very gregarious" and tend to swarm around the male of *Pyxicephalus* remaining in the water. Even juveniles have been known to form swarms (POYNTON, 1964). It would be interesting to understand the nature of these aggregations.

Tadpoles of various species recognize their siblings and distinguish tadpoles of different clutches. This behaviour has been particularly studied in the American toad *Bufo americanus* (WALDMAN, 1985). Factors that allow sibling recognition seem to be fixed or modified in ontogenetic development. A factor permitting sibling recognition might be transferred between the sibling tadpoles, or it might be transferred from the mother to her offspring. Differential recognition of maternal half-siblings by separately reared tadpoles suggests contribution of a factor of the maternal parent (product of oviduct for example) (WALDMAN, 1981). The secretion of female femoral glands might be such a primary factor for *Aubria* and *Pyxicephalus* offsprings. It would provide a basis for learning the sibling smell chemical characteristics. This gland secretion might be deposited by the mother on the eggs while laying them and gland function might be determined by the ovulatory hormone system This hypothesis is more congruent with having active glands in females than the traditional hypothesis of stimulatory function during amplexus. Another kind of

"sexual character reversal" in anurans is known in *Limnodynastes peroni* (Myobatrachidae, Limnodynastinae), where females bear lateral fringes on the fingers (DUELLMAN & TRUEB, 1985: 56-57): in this species, females use their hand in paddling movements for stirring water and spawn into a foam nest. The use of a secondary sexual character in offspring care would be an interesting parallelism. Experimental investigations could be carried out on *Aubria* and *Pyxicephalus* to test the hypothesis of chemical transmission of family recognition.

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Site fidelity and homing ability in *Hyla labialis* (Anura, Hylidae)

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A Colombian highland population of *Hyla labialis* was studied for 73 consecutive weeks by applying a unique toe clip combination to each of 304 adult male and 181 adult female individuals captured and released in the field. The analysis of a recapture record of 74 % for males and 43 % for females revealed that frogs released at their capture site were reencountered almost exclusively there (98 % males, 89 % females), hence demonstrating site fidelity. The majority of frogs translocated to a release site at a distance of up to 200 metres returned to their original capture site (58 % males, 53 % females), hence demonstrating homing ability. The frogs' spatial behaviour varied in relation to the breeding cycle of the population. After translocation between two successive breeding seasons, most males as well as females homed. When translocated during the breeding season, relatively more males homed and relatively more females remained at the foreign release site. Almost 30 % of all translocated frogs remained at the foreign release site and about 15 % were found at a third site. The short-term reaction to translocation was stronger in males (53 % homed) than in females (31 % homed), but as time passed the discrepancies leveled off and a year later no statistically significant differences remained between male and female spatial behaviour.

INTRODUCTION

Short or long lasting site fidelity and homing ability have been reported for several species of anuran amphibians belonging to different families (review in SINSCH, 1992*b*). Site fidelity may be related to stationary behaviour of territorial or brood-caring individuals (MCVEY et al., 1981; STEWART & RAND, 1992; VAN WIJNGAARDEN & VAN GOOL, 1994), but in many cases seasonal migratory long distance movements are involved, where each individual exhibits its site fidelity and homing ability through active round trips between two or more places (breeding site, estivation site, hibernation site), carried out in a repetitive manner according to the reproductive cycle of the population (HEUSSER, 1968; GLANDT, 1986; SINSCH, 1990).

Site fidelity and homing ability have also been demonstrated experimentally by displacing individual frogs from their capture site to a different place at a certain distance, where they were released and either followed in order to see where they were heading (OLDHAM, 1966; TRACY & DOLE, 1969; SINSCH, 1988) or marked with the expectation

of recapture near their original capture site (SINSCH, 1990; RITKE et al. 1991; PAPI, 1992).

Most of these studies on site fidelity and homing ability have been conducted in the temperate zones of Europe (HEUSSER, 1969; RYSER, 1989; SINSCH, 1992*a*) and North America (OLDHAM, 1966; DOLE, 1972). However, these faculties have also been revealed for some neotropical frog species (DIXON & STATON, 1976, MCVEY et al., 1981; CRUMP, 1986; SINSCH, 1988; STEWART & RAND, 1992; VAN WIJNGAARDEN & VAN GOOL, 1994).

The purpose of the present study was to examine site fidelity and homing ability in the neotropical frog species *Hyla labialis*. In this paper I report the results of an experiment designed to survey the spatial behaviour of this high mountain frog and to analyse it in relation to gender and reproductive activity of the population.

MATERIAL AND METHODS

STUDY AREA

The field study was carried out in the Parque Nacional Natural Chingaza nature reserve at 3500 metres in the eastern chain of the Andes mountains near the Colombian capital Santafé de Bogotá. This páramo is open grassland interspersed with spongy moss cushions and rosette plants (VUILLEUMIER & MONASTERIO, 1986). The ground is bog-like in many parts. Higher vegetation is sparse and patchy, consisting of small-leafed shrubs and the characteristic tall frailejones (several species of the genus *Espeletia*, family Compositae). The climate at this high altitude is cold and humid. There is a strong daily but only a small yearly temperature cycle. Daytime air temperatures may rise to 22°C when it is sunny, but mostly stay below 12°C, due to the usually heavy cloud cover and frequent rains. Nightly frosts occur occasionally, particularly in the dry season (December to March). The precipitation pattern in Chingaza is unimodal, peaking in June with about 260 mm of a total yearly rainfall of 1900 mm (SARMIENTO, 1986).

Hyla labialis was studied in a valley that stretches and slopes gently in a north-south direction for about 700 metres, where six groups of shallow ponds 100-200 metres apart (identified by the letters C, I, V, B, P and L) offered suitable breeding sites for this species (fig. 1). *H. labialis* was the only frog species that used these ponds for egg deposition. The only other frog species present in the study area, *Colostethus subpunctatus*, *Eleutherodactylus bogotensis*, and *E. elegans*, deposit terrestrial eggs.

BREEDING BIOLOGY OF *HYLA LABIALIS*

These frogs (male mean SVL 51 mm, female mean SVL 63 mm) usually breed in permanent ponds. Amplexus occurs in the water, where calling males are approached by receptive females. Whereas males spend several months per year in or near the breeding pond, females enter the water mainly for oviposition, which usually takes one night.

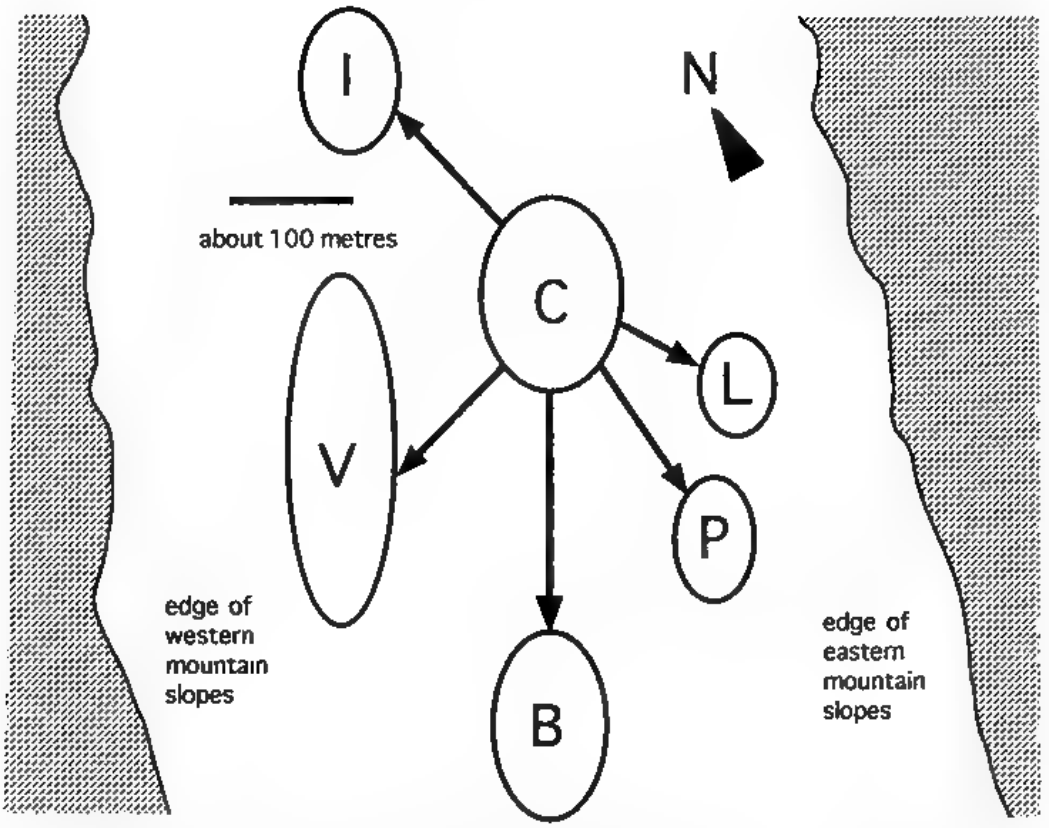

Fig. 1. – Sketch of the mountain valley where the spatial behaviour of *Hyla labialis* was studied. Control frogs were captured and released in the central breeding area C. Experimental frogs from five breeding areas (I, V, B, P and L) were translocated to area C and released there. Arrows indicate the homeward directions and rough distances.

Females spend most of their life on land, up to hundreds of metres away from the water. Two main breeding seasons per year last from about one to three months each: one peaks in February-March, the other in September-October. Occasional breeding activity may occur any time, even during the driest months of the year, when there is little water in the ponds.

Testing for site fidelity and homing ability

From June 1991 until August 1992, the six breeding areas were searched during daylight hours for adult *H. labialis* about once a week by slowly walking around the ponds in order to spot frogs visually. All encountered frogs were captured and censused. Many of these animals were already individually marked by toe-clipping from previous studies (Lüddecke, unpublished). All newly caught frogs were marked individually in the same manner. Most animals were released on the same day of capture, but some were kept in the laboratory for a week prior to their release. The search for marked frogs was continued until December 1992, but after August 1992 all encountered animals were released at their capture site.

To test for site fidelity, 187 frogs found in the central breeding area C were released at their capture site. To determine their homing ability, 298 individuals captured in the five breeding areas surrounding area C were translocated to area C and released there. Recapture of these translocated frogs at their original capture site would indicate homing behaviour.

Statistical analyses

Due to disparate amounts of data for males and females, and lack of normal distribution of data, many comparisons were made after converting actual counts into percentage numbers, or applying chi-square tests or non-parametric statistics (Neave & Worthington, 1988).

Results

Recaptures

A total of 485 individual adult *Hyla labialis* were marked and released. Of these, 306 individuals were recaptured, yielding an overall recapture rate of 63.1 %. Recapture rates differed significantly between males and females ($\chi^2 = 45.6$, $P < 0.01$). In both sexes, recapture rates were slightly higher for translocated frogs than for home-released frogs (Table I). Recapture success was highest during peaks of breeding activity when frogs were abundant at the ponds. Time intervals between capture and first recapture ranged from one week to 73 weeks. The mean time interval between release and first recapture was shorter for males (18 weeks) than for females (23 weeks). During 26 weeks after initial release, the time span between two reproductive peaks, relatively more males (167 of 227, 74 %) than females (46 of 79, 58 %) were recaptured.

Site fidelity

Of 86 individually marked males originally captured and released in the central breeding area C, 85 (98.8 %) were recaptured in the same area. The recapture rate for females (25 of 28, 89.3 %) at the original capture site was not significantly different from that of males ($\chi^2 = 3.22$, $P > 0.05$). Four frogs left the home area and each moved to a different site.

Homing ability

Because there were no significant differences in homing between frogs from the five breeding areas surrounding C ($\chi^2 = 5.15$, $P > 0.05$), I pooled the data from these areas

Table I. - Recapture record of individual adult male and female *Hyla labialis* handled in site fidelity and homing ability experiments in the Páramo de Chingaza.

Treatment	Males		Females	
	N	%	N	%
Released at capture site	86 of 119	72.3	28 of 68	41.2
Released after translocation to another site	141 of 185	76.2	51 of 113	45.1

Table II. - Long-term spacial behaviour of 141 adult male and 51 adult female *Hyla labialis* after translocation from their original capture site to a common central release site.

	Males		Females	
	N	%	N	%
Returned to original capture site	82	58.1	27	52.9
Remained at release site	42	29.8	14	27.4
Moved to third site	17	12.1	10	19.6

Table III. - Comparison of long-term spatial behaviour during and between breeding seasons of adult male and female *Hyla labialis* after capture and release in breeding area C and after translocation to C from all other breeding areas combined.

A. Breeding area C.

	During breeding seasons				Between breeding seasons			
	Males		Females		Males		Females	
	N	%	N	%	N	%	N	%
Site specific	46	98	17	89	38	97	8	89
Moved to another site	1	2	2	11	1	3	1	11

B. All other breeding areas combined.

	During breeding seasons				Between breeding seasons			
	Males		Females		Males		Females	
	N	%	N	%	N	%	N	%
Returned to capture site	35	55	16	52	47	61	11	55
Remained at release site	21	33	7	22	21	27	7	35
Moved to third site	8	12	8	26	9	12	2	10

(Table II). The majority of the frogs recaptured after translocation had returned to their original capture site. Some individuals homed within a single week, others were not seen again until more than a year later. Many translocated frogs were recaptured at the release site and others were found at a third site. The spatial behaviour of translocated frogs was significantly different from random (Goodness-of-fit test, $\chi^2 = 53.7$, $P < 0.001$). Both sexes showed the same general tendency and, in spite of some disparities, there was no significant difference between male and female spatial behaviour ($\chi^2 = 1.76$, $P > 0.05$).

Spatial behaviour in relation to reproductive cycle

During the study period there were three reproductive peaks: October 1991, February 1992, and October 1992. The approximate length of each breeding season was determined by the presence of amplectant pairs and recently deposited egg clusters of *H. labialis* in the breeding ponds. Over the entire study period, males and females from the central breeding area C were equally site-specific, regardless of being released during or between breeding seasons (Table III).

To estimate the spatial behaviour of translocated frogs in relation to breeding activity, as a first approach I used first-recapture data covering the entire study period in order to look for long-term patterns (Table III). There were only slight and insignificant differences ($\chi^2 - 0.41$, $P > 0.05$) in male behaviour. a few less homed and a few more remained after translocation during a breeding season compared to translocation between breeding seasons. Females homed about equally well anytime and performed similarly to homing males. Considerably fewer females remained at the foreign release site and instead moved to a third site when translocated during the breeding season compared to their behaviour after translocation between breeding seasons. Due to this shift, there was a highly significant behavioural difference between females translocated during or between breeding seasons ($\chi^2 = 17.4$, $P < 0.01$).

In a second approach I used multiple-recapture data and set a time limit of ten weeks between release and recapture This 10-week interval was presumably short enough to ensure that frogs had not yet entered their next reproductive phase. The males' short-term reaction was the same during and between breeding seasons. In contrast, female behaviour differed according to season: when translocated during a breeding season, more than half of the females remained at the foreign release site, but when translocated between breeding seasons, more than half of the females homed (fig. 2). Again, females behaved differently ($\chi^2 = 8.9$, $P < 0.01$) depending on the timing of translocation, but this time the shift occurred between returning and remaining frogs. Few males and females moved to a third site.

Gradual change in spatial behaviour

Having found differences between the frogs' short- and long-term reactions to translocation, I examined how the spatial behaviour changed over time I processed first-recapture data obtained from a sample of 110 individual frogs (86 males, 24 females)

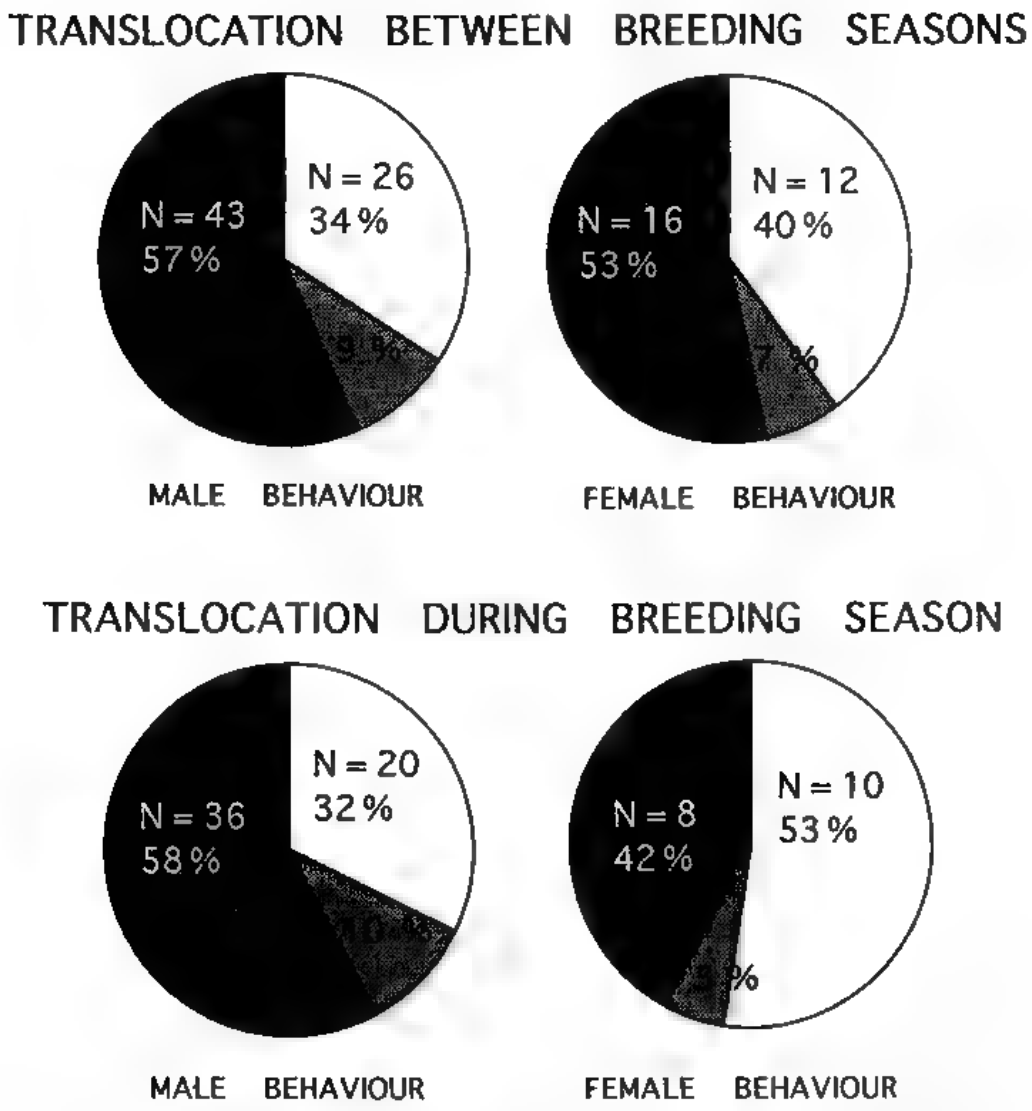

Fig. 2. – Spatial behaviour of male and female *Hyla labialis* within the first ten weeks after translocation either between or during breeding seasons, based on multiple recapture data Black areas: frogs returned to capture site; grey areas: frogs moved to third site; white areas: frogs remained at release site.

captured during the same breeding season in the breeding areas surrounding C, translocated and released at C. When calculating the proportion of frogs in each behavioural category by accumulating data from sequential recapture-samples obtained first at the end of that breeding season (week 14), and afterwards at ten-week intervals, a gradual and statistically highly significant (Friedman Two-Way ANOVA, $\chi^2 = 11.07$, $P = 0.003$) change in the proportions of frogs in each behavioural category became evident as more and more frogs were recaptured (fig. 3).

Initially there was a large difference between the sexes: more than half of the males, but only one third of the females had homed. In contrast, proportionately more females

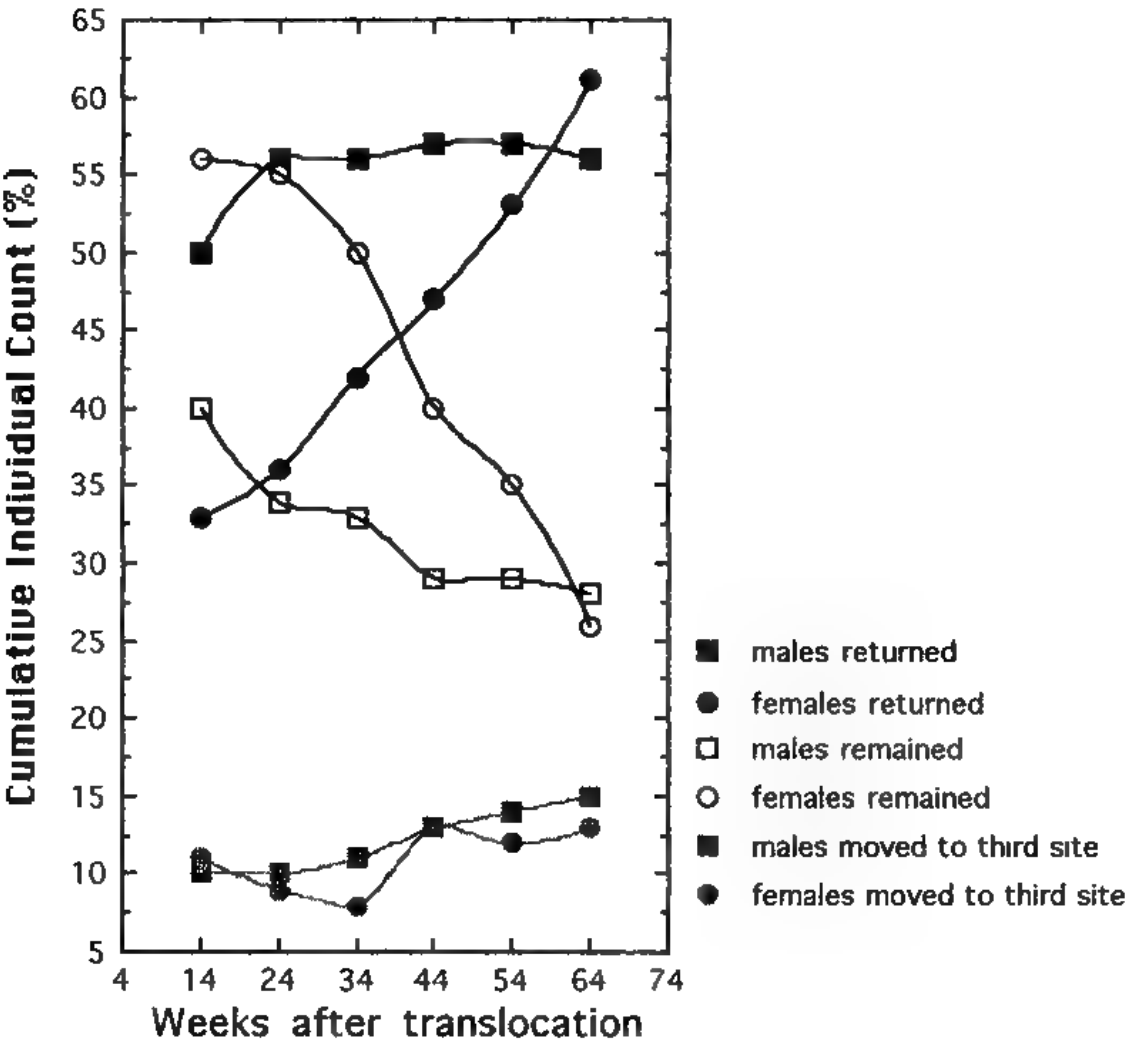

Fig 3 – Temporal change in spatial behaviour of 110 adult male and female *Hyla labialis* translocated in the same breeding season.

than males remained at the foreign release site during the first weeks after translocation. The relative numbers of males and females that moved to a third site were about equal. Almost six months later (week 24), males had attained a distribution approaching the final one, whereas that of females was still close to the initial one. However, when all individuals had been recaptured, the differences between males and females had almost vanished, and the situation resembled the long-term result for all translocated males and females (Table II) But the patterns of behavioural change over time differed significantly between sexes (match test $M_1 = 30$, $P < 0.05$).

DISCUSSION

RECAPTURE RECORD

Comparison of recapture rates reported for anuran species is difficult due to differences in capture schedule, search intensity and marking techniques, and conspicuousness, activity cycles and abundance of the species studied. With values over 70 % for males and over 40 % for females, the recapture rates for *Hyla labialis* were high compared with those reported for other anuran species (OLDHAM, 1966; HOTZ, 1970; RITKE et al. 1991; BUCHACHER, 1993). This may be related to my continuous capture-recapture programme over a period of 73 consecutive weeks, whereas most other studies relied on one capture period in the first year and a recapture period about one year later, both timed during breeding activity (OLDHAM, 1966; TRACY & DOLE, 1969; DOLE, 1972; RITKE et al. 1991; SINSCH, 1992*a*).

SITE FIDELITY

Male as well as female *H. labialis* showed a high degree of site fidelity, similar to the results obtained for *H. chrysoscelis* (RITKE et al., 1991), *Bufo americanus* (OLDHAM, 1966), and male *B. calamita* (SINSCH, 1992*a*). BUCHACHER (1993) obtained a lower site-fidelity rate of 67 % for *Pipa arrabali*, which he ascribed to a high mobility of individuals between adjacent ponds that were only a few metres apart. Remarkable cases of site specificity, where individuals were recaptured only a few metres distant from the original capture site, have been reported for male *Bufo bufo spinosus* in Italy (HOTZ, 1970), *Leptodactylus macrosternum* during the wet season in Venezuela (DIXON & STATON, 1976), and some territorial dendrobatid species (MCVEY et al. 1981; VAN WIJNGAARDEN & VAN GOOL, 1994). This precision in site specificity was also observed in this study for some individual males and females of *H. labialis*

The absence of site fidelity in *Bufo verrucosissimus* has been ascribed to the instability of suitable spawning sites in successive years (TARKHNISHVILI, 1994), and in *B. calamita* females to their opportunistic choice of the spawning site in response to calling males (SINSCH, 1992*a*). The strong site fidelity of *Hyla labialis* females conforms to the environmental conditions and reproductive biology of the population studied. Although long-term pond stability in the páramo is undocumented, the breeding ponds in the study area did not dry for several years, even during the strong El Niño year of 1992 (LÜDDECKE, personal observation). Thus site-specific females seem to run almost no risk in moving to a home pond and finding it dry. Because spawning may happen occasionally almost any month of the year, female *H. labialis* also benefit from being site-specific when the scarcity of males at the breeding ponds would offer little opportunity for long-distance phonotactic orientation to callers.

Homing tendency

Translocated adult *H. labialis* were recaptured at the breeding ponds mostly during the breeding season, indicating that frogs returned there for reproduction. The moderate percentage of homing *H. labialis* (56.8 %) probably was not due to increased mortality while moving across the terrain, since the recapture rates of translocated frogs were higher than those of home-site released frogs. If frogs recaptured at a third site are regarded as potentially homing, then about 70 % of the translocated individuals had this tendency. This interpretation seems justified by the fact that some translocated frogs detoured to a third site prior to returning to the home site. Another possible reason for the moderate homing success, in spite of the strong homing tendency, is related to the high site fidelity evidenced by the control group: a strongly site-specific individual (although it may migrate) could be familiar with just a small fraction of the entire valley that was used as the study area. This would mean that finding home after displacement was hampered in individuals whose release site lay outside their migratory corridor. Nothing is known about the orientation mechanisms used by *H. labialis*.

Almost 30 % of the translocated and recaptured *H. labialis* remained at the foreign release site. One possible reason for this behaviour is that the release site (breeding area C) was an appropriate place for reproduction and was therefore accepted in exchange for the original site. Most remaining frogs were recaptured only once shortly after release and their long-term spatial behaviour is unknown. However, some frogs first recaptured at the release site were found at the original capture site on their second or third recapture, indicating that they had only delayed their homeward journey.

Ritke et al. (1991) pointed out that long intervals between release and recapture may be due to slow recovery from a reproductive effort. The sex difference in recapture rate of *H labialis* translocated in one and recaptured in the next breeding season implies that about half of the females skipped every other breeding season and spawned only once per year, while most males participated in every breeding season. This would indicate that females recovered slower than males from a reproductive event.

Since shortly after translocation during the breeding season most females were still found at the release site (fig. 3), at first glance this would mean indifference as to where to oviposit. But it turned out that, when recaptured, most of these females had not yet spawned at the foreign release site. Half of the females found at a third site and most females that had already returned to their home site when recaptured shortly after translocation were still gravid (Lüddecke, unpublished data). These results are compatible with strong site fidelity and suggest that females have the tendency to oviposit at a familiar breeding site.

Resumen

Una población de *Hyla labialis* de alta montaña en Colombia fue estudiada durante 73 semanas consecutivas, aplicando un marcaje único a cada uno de 304 machos y

181 hembras adultos capturados y liberados en el campo. El análisis de los datos de recaptura del 74 % de machos y 43 % de hembras reveló que las ranas capturadas y liberadas en su sitio de captura fueron reencontradas casi exclusivamente allí (98 % de los machos, 89 % de las hembras), lo que demostró su fidelidad al hogar. La mayoría de las ranas traslocadas a un sitio de liberación a una distancia de hasta 200 metros regresó a su sitio de captura original (58 % de los machos, 53 % de las hembras), lo que demostró su capacidad de retornar al hogar. El comportamiento espacial de las ranas variaba acorde al ciclo reproductivo de la población: después de la translocación entre dos épocas reproductivas sucesivas, la mayoría de los machos y hembras retornaban al hogar. Después de una translocación durante una época reproductiva, relativamente más machos retornaban, pero relativamente más hembras se quedaban en el sitio de liberación. Casi el 30 % de las ranas translocadas se quedaba en el sitio de liberación y el 15 % fue encontrada en otro sitio distinto. La reacción inmediata a la translocación era más fuerte en los machos que en las hembras (53 % y 31 %, respectivamente, regresaban al sitio de captura), pero con el paso del tiempo las discrepancias en el comportamiento espacial disminuyeron y un año después no quedaron diferencias significativas entre machos y hembras.

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Merocrine secretion from serous cutaneous glands in *Rana esculenta* complex and *Rana iberica*

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Serous cutaneous glands of larval and juvenile specimens pertaining to the *Rana esculenta* complex and *Rana iberica* may modulate product discharge through exocytotic release and weak compression from the myoepithelial sheath. This method of secretory release is a typically merocrine mechanism and is an unusual functional trait in these glands, which are generally credited with holocrine bulk discharge of anti-predatory poison products. Current trends in animal toxinology claim that the most common active compounds in anuran poisons (i.e. biogenic amines and peptides) played an early role in skin homeostasis during the phylogeny of this order, before participating in chemical skin defence against predators. On the basis of this hypothesis, we conclude that the merocrine activity described in *Rana* serous cutaneous glands is an ancestral functional characteristic related to the use of skin products in local regulative mechanisms.

INTRODUCTION

The concept of holocrine secretory activity performed by serous glands in anuran skin was developed by FARAGGIANA (1938*b*), who carried out pioneering experimental studies on the regenerative processes which follow glandular discharge (FARAGGIANA, 1938*a*, 1939). Her findings were later confirmed under TEM (DELFINO, 1980), and further investigations, coupled with pharmacological approaches (BENSON & HADLEY, 1969; HOLMES et al., 1977; HOLMES & BALLS, 1978; DELFINO et al., 1982), stressed the role of the myoepithelial cells in the massive release of the serous product together with the secretory cytoplasm and nuclei.

The latter method of secretory discharge is consistent with the use of the serous secretory products as anti-predatory toxins and/or repellents (DUELLMAN & TRUEB, 1985). Nonetheless, recent studies on the *Rana esculenta* complex, showing morphological changes in the granules containing serous skin products, suggested that these substances are also employed in skin homeostasis (BARNI et al., 1987) Furthermore, ultrastructural evidence has proved that discrete amounts of product are released by serous glands in juvenile *Hyla arborea* through exocytosis (DELFINO et. al., 1994). These findings disclose

new perspectives to the interpretation, both functional and evolutive, of serous skin secretion in anurans (DALY et al., 1987). In the light of this updated approach to the regulating function of poison secretions of anuran skin, we aimed to investigate whether the serous cutaneous glands of two groups of European frogs, the green frogs (Italian *Rana esculenta* complex) and the brown frogs (Spanish *Rana iberica*), can release their cutaneous serous products through exocytosis, i. e. by merocrine mechanisms. The above taxa were studied as they represent a genus which includes extensively studied species, both morphologically and physiologically, commonly found in laboratories. This choice enabled us to obtain adequate references from previous literature and at the same time allows verification of our results.

We avoided any conditions which could elicit bulk secretion and focused on the patterns which could refer to the modulated release of the serous product. We observed advanced larval specimens and newly metamorphosed froglets, as these development stages appeared to best fit our purpose. Serous glands in late tadpoles show advanced patterns of biosynthesis but relatively undifferentiated myoepithelial cells, a condition which may *minimize* bulk discharge; on the other hand, juveniles are very imperfect terrestrial vertebrates and must *maximize* their cutaneous homeostatic mechanisms when exposed to the subaerial environment.

We detected evidence of merocrine secretory processes and compared them with the traditional holocrine mechanism reported in serous cutaneous glands of anurans.

MATERIAL AND METHODS

Larval specimens of Italian green frogs (*Rana esculenta* complex) and of the Iberian brown frog *Rana iberica* were collected from the outskirts of Florence (Italy) and Mellid (Santiago de Compostela, Spain), respectively. The tadpoles were reared in the laboratory until the first specimens underwent metamorphosis. The schedule below reports the developmental range considered, according to TAYLOR & KOLLROS (1946), as well as the number of animals and the samples observed for each, under light (LM) and electron microscopes (TEM).

(A) Stage XX, *Rana esculenta* complex: LM, 3 tadpoles, 2 samples from each; TEM, 2 tadpoles, 2 samples from each.

(B) Stage XXII, *Rana iberica*: LM, 3 tadpoles, 3 samples from each; TEM, 3 tadpoles, 3 samples from each.

(C) Stage XXV (juvenile), *Rana esculenta* complex: LM, 3 froglets, 2 samples from each; TEM, 2 froglets, 2 samples from each.

(D) Stage XXV, (juvenile) *Rana iberica*: LM, 2 froglets, 2 samples from each; TEM, 2 froglets, 2 samples from each.

The choice of the developmental range was based on former studies which showed that, between stages XIX and XXV, anuran skin possesses small, but already differentiated glands which already contain large secretory accumulations undergoing post-Golgian maturation (DELFINO, 1977; DELFINO et al., 1988, 1994). Furthermore, this developmental

range also embraces the differentiation timing of the glandular myoepithelial sheath (DELFINO et al., 1987), which plays a role in gland depletion.

Tadpoles and froglets were anaesthesized in 0.2 % aqueous chlorbutol and sacrificed. Small bands of skin (about 4 mm^2) were removed from the back and fixed in Karnovsky's aldehyde mixture (KARNOVSKY, 1965). The skin strips were then reduced into smaller (about 2 mm^2) fragments and post-fixed in 1 % OsO_4. For all these procedures, a 0.1 M, pH 7 sodium cacodylate buffer was employed, at a temperature of 4°C. The specimens were dehydrated in a graded ethanol series, soaked in propylene oxide and infiltrated with Epon 812 to obtain flat embeddings. The embeddings were then cut with a NOVA LKB ultramicrotome into semithin (1-1.5 µm) and ultrathin (silver-white interference colour) sections, these sections were used for the light and electron microscope observations, respectively. The semithin sections were stained with buffered 10 % toluidine and used to prepare ultrastructural investigation. The ultrathin sections were collected on uncoated copper grids and stained with a hydroalcholic saturated solution of uranyl acetate, followed by bismuth subnitrate (0.8 mg/ml in an alkaline solution). These specimens were finally observed under a Siemens 101 electron microscope at 80 kV.

RESULTS

Under the light microscope, serous glands at premetamorphic stages XX and XXII (fig. 1a-b) exhibit the structural feature characteristic of the Anura. The secretory units, which are syncytial in structure, are provided with a continuous contractile sheath of myoblasts (the future myoepithelial cells, mec) as well as a cap of undifferentiated cells, representing the regenerative matrix (intercalary tract or neck) of the gland. According to the usual ultrastructural patterns shared by all serous glands of anurans, the poison adenomeres of *Rana* tadpoles possess exiguous lumina, which are restricted to the subintercalary level. However, in advanced larvae of the Iberian frog, these cavities are rather enlarged (fig. 1a-b) when compared to other anurans studied so far. The luminal space sinks into the secretory syncytium and resembles a multichambered compartment, owing to the interposition of slender cytoplasmic walls, which look like thin bridges between the secretory unit and neck (fig. 1a-b). Observation of serial sections revealed that duct and gland lumina are separated only by a discontinuous cytoplasmic screen (fig. 1b). Actually, the intercalated cells, which are arranged in an irregular doughnut structure, partly obstruct the central opening with slender cytoplasm projections. The secretory product may reach the duct lumen through the spaces separating these cell processes. Secretory granules crowd around the luminal boundary and mould themselves around its surface, so that they assume a crescent-like shape in section.

In juvenile glands, the serous syncytium is a solid structure, thus limiting the small lumen to the neck region (fig. 1c). The secretory cytoplasm holds large amounts of poison product, which consists of dense particles, subspherical in shape. However, larger magnifications revealed a spongy-like substructure in some granules (fig. 1d).

Under the electron microscope, the secretory syncytium possesses a well developed biosynthesis apparatus and nuclei scattered in a single row at the periphery. Slender rough

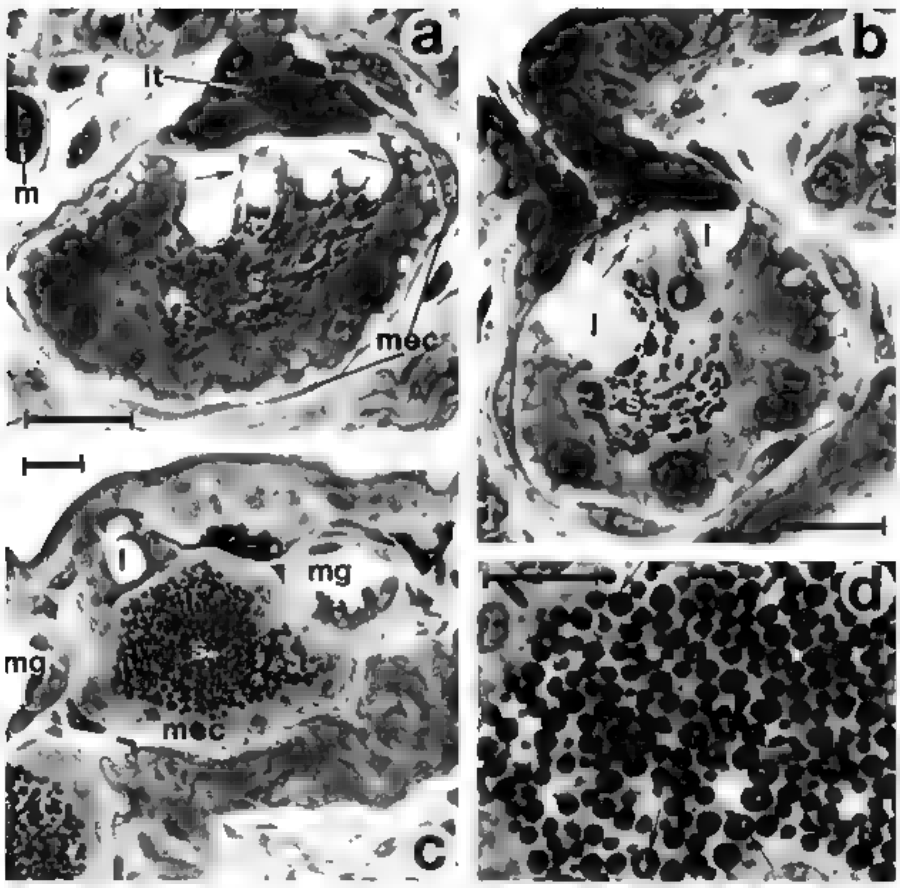

Fig. 1. — Serous gland anlagen under light microscope. Bars: 10 µm.

a. — *Rana iberica* (XXII). Longitudinal section tangential to the intercalary tract (it), consisting of a few layers of undifferentiated cells. The apical portion of the secretory syncytium displays a multichambered lumen with slender cytoplasmic partitions (arrows). Notice the serous secretory product (s) crowded around the lumen and the thin myoepithelial cell layer (mec). m: melanophore.

b. — *Rana iberica* (XXII). Longitudinal section through the widest diameter of the secretory unit, as shown by the lumen of the duct which is obvious. Only a thin, discontinuous screen (arrowhead), formed by slender cytoplasmic processes of intercalary tract cells, separates the duct and adenomere lumina. Coupled arrows in the duct outlet, arrow points to cytoplasmic partition between apical chambers. s: serous secretory product.

c. — *Rana esculenta* complex (juvenile). Longitudinal section: the secretory unit lacks any obvious lumen, a slender cavity (l) is detectable only in the intercalated tract-duct complex. Note two mucous glands (mg) provided with a wide lumen. mec: myoepithelial cell layer; s: serous secretory product.

d. — *Rana esculenta* complex (juvenile). Detail of the serous secretory product contained in the syncytial cytoplasm. Arrows point to granules with a spongy-like substructure.

endoplasmic reticulum (rer) cisterns (fig. 2a-b) and Golgi stacks (dictyosomes) (fig. 2a, 2c-d) are consistent in both species observed, and occupy the peripheral cytoplasm. The distal (trans) face of the Golgi stacks dispatches minute vesicles (fig. 2d), which merge together to form wide, up to 4 μm in diameter, secretory deposits. These large vesicles contain a finely dispersed product (fig. 2c-e, 5a), which undergoes marked condensation (maturation) in later stages of biosynthesis (fig. 3a). Several merging processes affect the secretion aggregates (fig. 3b, 4), which sometimes display a spongy-like substructure, owing to the alternation of darker and paler zones. Secretory product maturation is a gradual process in which the granules become involved at different rates. The process produces a remarkable polymorphism, due to the coexistence of intermediate stages of condensation. In some instances the intermediate steps are by-passed, as revealed by condensation patterns affecting the material freshly dispatched from the Golgi stacks (fig. 3c).

During the condensation phase, the membrane which borders the serous aggregates becomes detached from the secretory material and leaves a transparent halo around it In the meantime, numerous, slender (about 25 nm in diameter), microvilli-like outgrowths of the cytoplasm intrude this perigranular space. The microvilli are branched and hollow, and form a network around the secretory product (fig. 3d).

From the central cytoplasm the secretory granules reach the upper level of the syncytium, just beneath the lower cell layer of the intercalary tract (neck) of the gland. As observed under light microscope, a proper lumen exists at this level, separated from the neck and duct lumina only by interposition of slender cytoplasmic processes of the intercalated tract cells. The serous deposits crowd around the lumen (fig. 4) and some adhere to the luminal plasmalemma, so that their limiting membranes merge with it. Rather large openings form in this way, which allow the secretory product to be released into the lumen (fig. 4, inserts A and B), following the pattern of a merocrine process. This mechanism appears, however, to be quite peculiar; the spaces holding the granules engaged in the secretory release are continuous with the compartments containing other serous aggregates, due to serial confluences (fig. 4, insert B). In this way the secretory product may flow out toward the lumen, at a rate which depends on the number of granules involved in reciprocal confluence.

The secretory units engaged in this exocytotic activity possess the neuro-contractile apparatus typical of serous glands, consisting of neurites and myoepithelial cells (fig 5a). The thin axons are clearly recognizable as they contain parallel neurotubules (fig. 5b-d) and electron transparent vesicles in their endings (fig. 5c), whereas in the myocytes myofilaments occupy large portions of the cytoplasm, leaving two symmetrical zones at the nuclear poles to accommodate scanty organelles (fig. 5a). Nevertheless, this muscle-nerve cell machinery seems to be still immature, since myofilaments fail to fill the myocyte cytoplasm, and neuromuscular junctions are infrequent and resemble poorly specialized, occasional contacts (fig. 5b). In their erratic course, some neurites may also make contact with the secretory syncytium (fig. 5d).

Serous glands in recently metamorphosed froglets display large amounts of cytoplasm filled with dense aggregates, which arise from further condensation of the secretory deposits described in larval adenomeres. Despite their density, secretory granules are not homogeneous in aspect as they exhibit the spongy-like substructure (fig. 6a-b) already

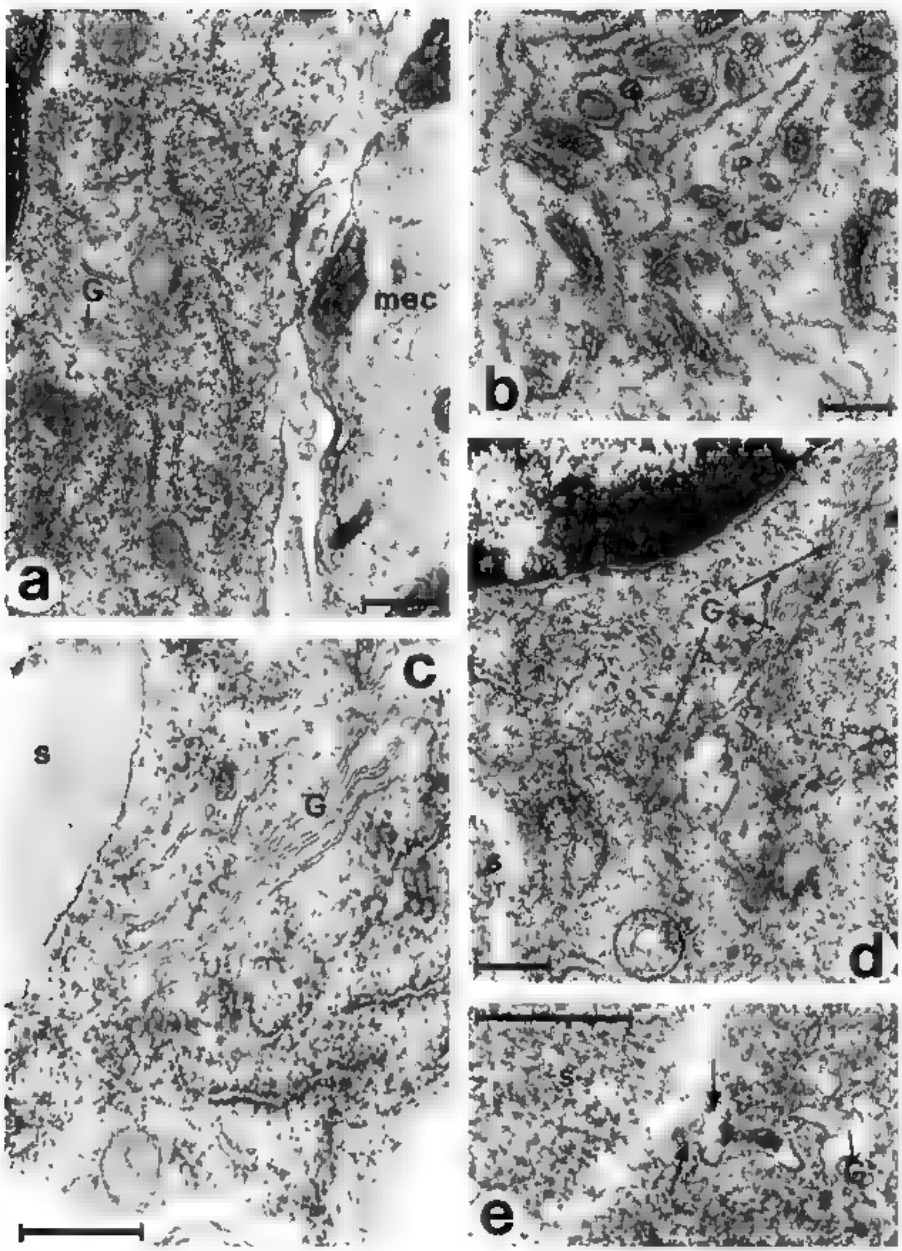

Fig. 2 – Biosynthesis apparatus in serous gland anlagen of both species. Bars 500 nm.

a. – *Rana esculenta* complex (XX). Peripheral portion of the secretory syncytium, closely contiguous to the myoepithelial layer. Note slender rer cisterns and a Golgi stack, or dictyosome (G). mec: myoepithelial cell.

b – *Rana iberica* (XXII) Parallel array of rough endoplasmic reticulum complements and mitochondria with dense matrix.

c. – *Rana esculenta* complex (XX). Golgi stacks (G) frequently occur in the perinuclear cytoplasm of the secretory syncytium The dictyosomes fulfil the activity of the rough endoplasmic reticulum and lead to the accumulation of a fine serous secretory material (s) inside large vesicles.

d-e – *Rana iberica* (XXII) Minute vesicles (encircled in d) derive from the periphery of the Golgi stacks (G) and merge (arrows in e) with the larger serous secretory deposits (s), contributing material to these storage structures.

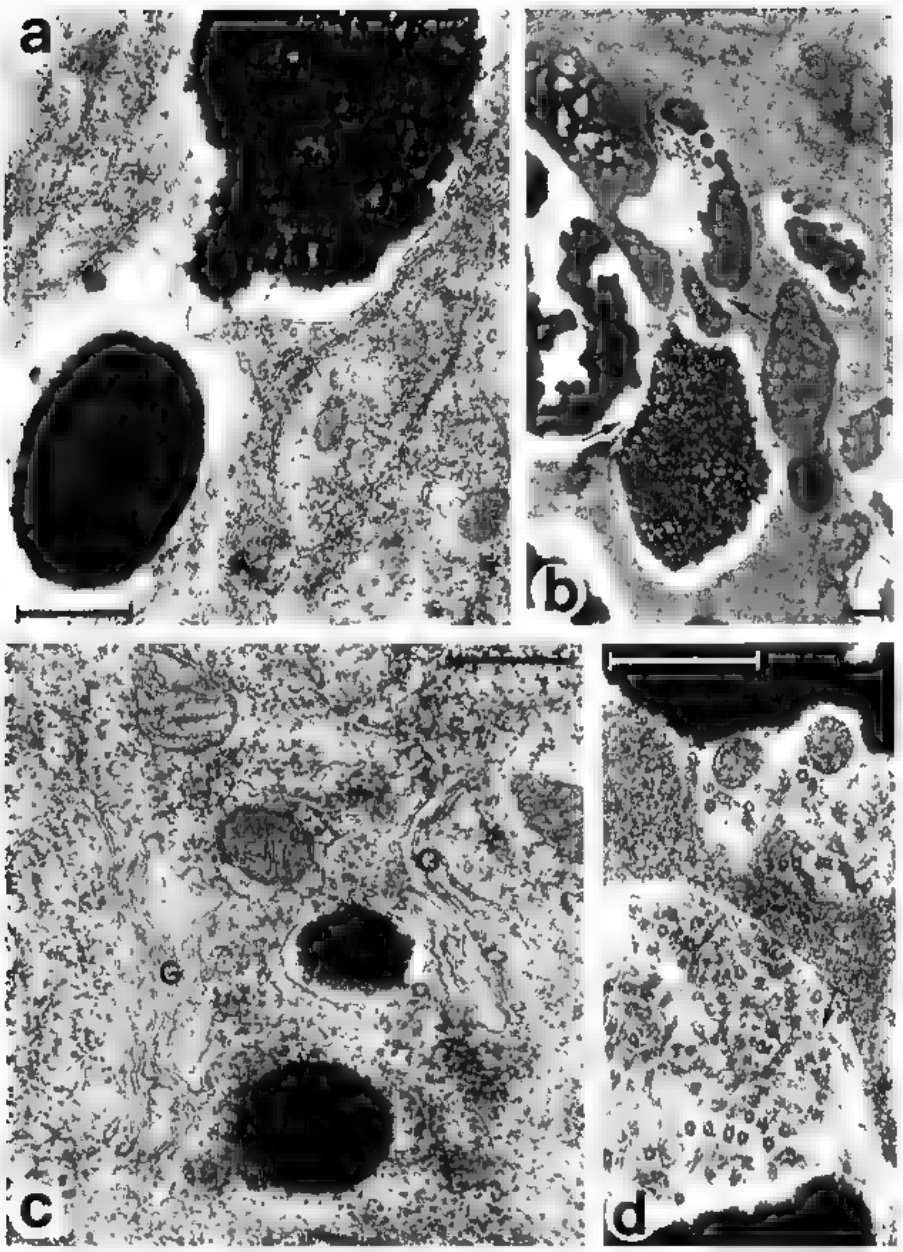

Fig. 3. – Patterns of poison maturation in both species. Bars: 500 nm.

a. – *Rana esculenta* complex (XX). The secretory product contained in the vesicles undergoes a remarkable condensation, which gives rise to granules provided with a compact substructure

b. – *Rana iberica* (XXII). Serous maturation, recognizable from variable density of the product, proceeds through sequential confluence processes between secretory aggregates (arrows).

c. – *Rana esculenta* complex (XX). In some instances the product contained in the vesicles dispatched by the Golgi stacks (G) is promptly condensed and the vesicle phase is by-passed

d. – *Rana iberica* (XXII). Slender microvilli fill the space between the limiting membrane and secretory product Arrows indicate branching points which give the microvillous cluster the appearance of a three-dimensional net.

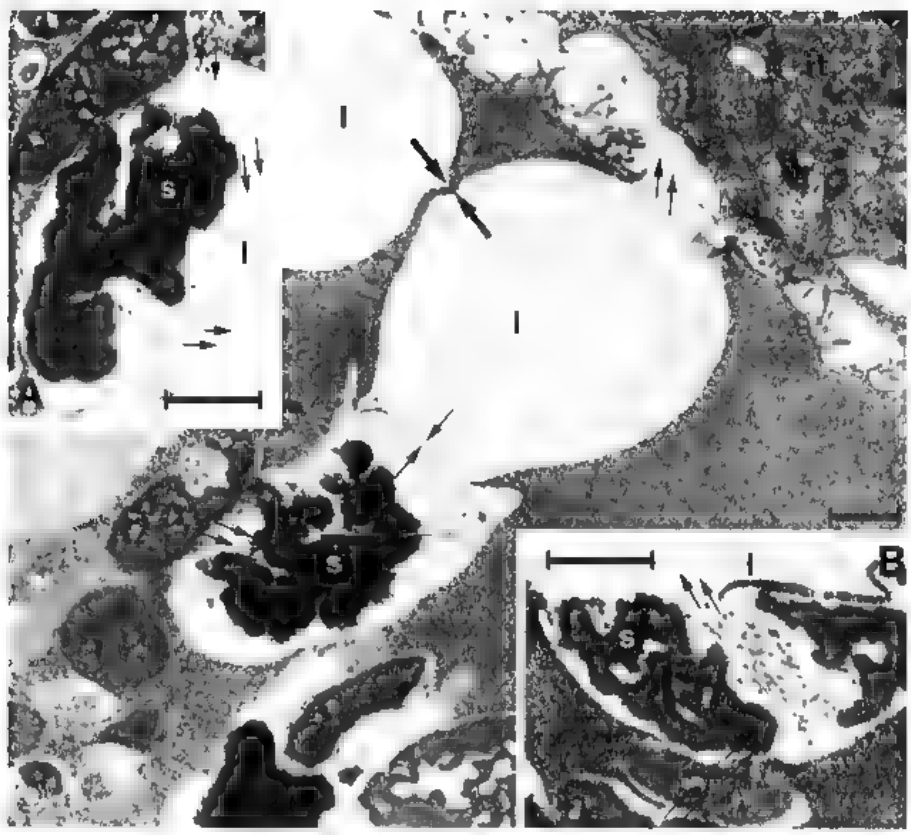

Fig. 4. – *Rana iberica* (XXII). Bars: 1 µm. Subintercalar portion of serous secretory unit. Note the multichambered lumen (l), the characteristic cytoplasmic walls (large arrows) and secretory aggregates of various density. Only an extremely thin screen (small arrows) separates the large vesicle from the lumen. Inserts A and B show continuity between vesicular and luminal compartments and between serous vesicles (B). Coupled arrows show outflow paths. it intercalary tract; s· serous secretory product.

described in the previous stages. In neometamorphosed froglets, the biosynthesis apparatus typical of protein manufacturing glands decays remarkably owing to rarefaction of rer and dictyosomes The organelles are first reduced to the very perinuclear zone and later disappear altogether (fig. 6a-b).

Touch stimulation of cutaneous areas, possibly painful in nature, may evoke local responses in the myoepithelial sheath of serous glands before sacrifice. Contracted myocytes show well-defined thickenings in their myofilament apparatus, which in turn cause remarkable morphological changes in the shape of the nuclei. Myofilaments act as a sphincter around the surface of the nucleus and mould it into the shape of an hourglass, with the peripheral half contained in the myocyte and the inner one bulging towards the secretory syncytium (fig. 6c).

The effects elicited by myocyte compression may be confirmed by patterns detectable in the gland duct. This is a slender intraepidermal interstice that crosses the epithelial

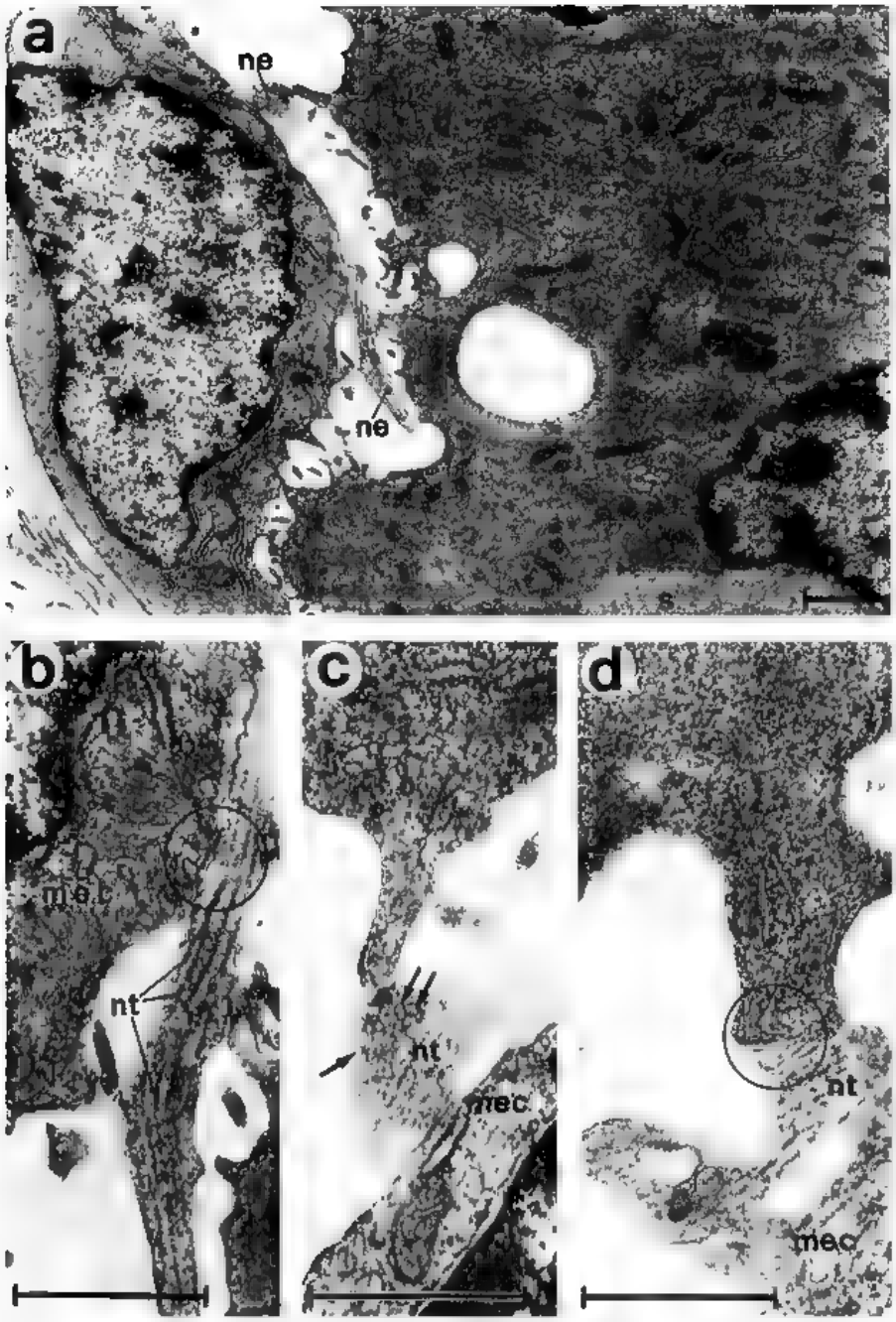

Fig. 5. *Rana iberica* (XXII). Bars: 1 μm. The neuromuscular apparatus of the serous gland anlagen consists of myoepithelial cells (mec) and neurites (ne). nt: neurotubules, s. serous secretory product

a. — Note the dome-shaped, perinuclear portion of the myoepithelial cell engaged in myofilament accumulation. The biosynthesis apparatus is obvious at the cell poles. Neurites are contained in the interstice between secretory and contractile compartments.

b. — Detail of the previous figure. Note parallel neurotubules in the axon and non-specialized contacts between neurite and myoepithelial cell (encircled).

c. — Detail of (a) showing a neurite ending. Small electrontransparent vesicles (arrows) are obvious

d. In some instances, only a 30 nm wide gap (encircled) separates the neurite from the serous syncytium.

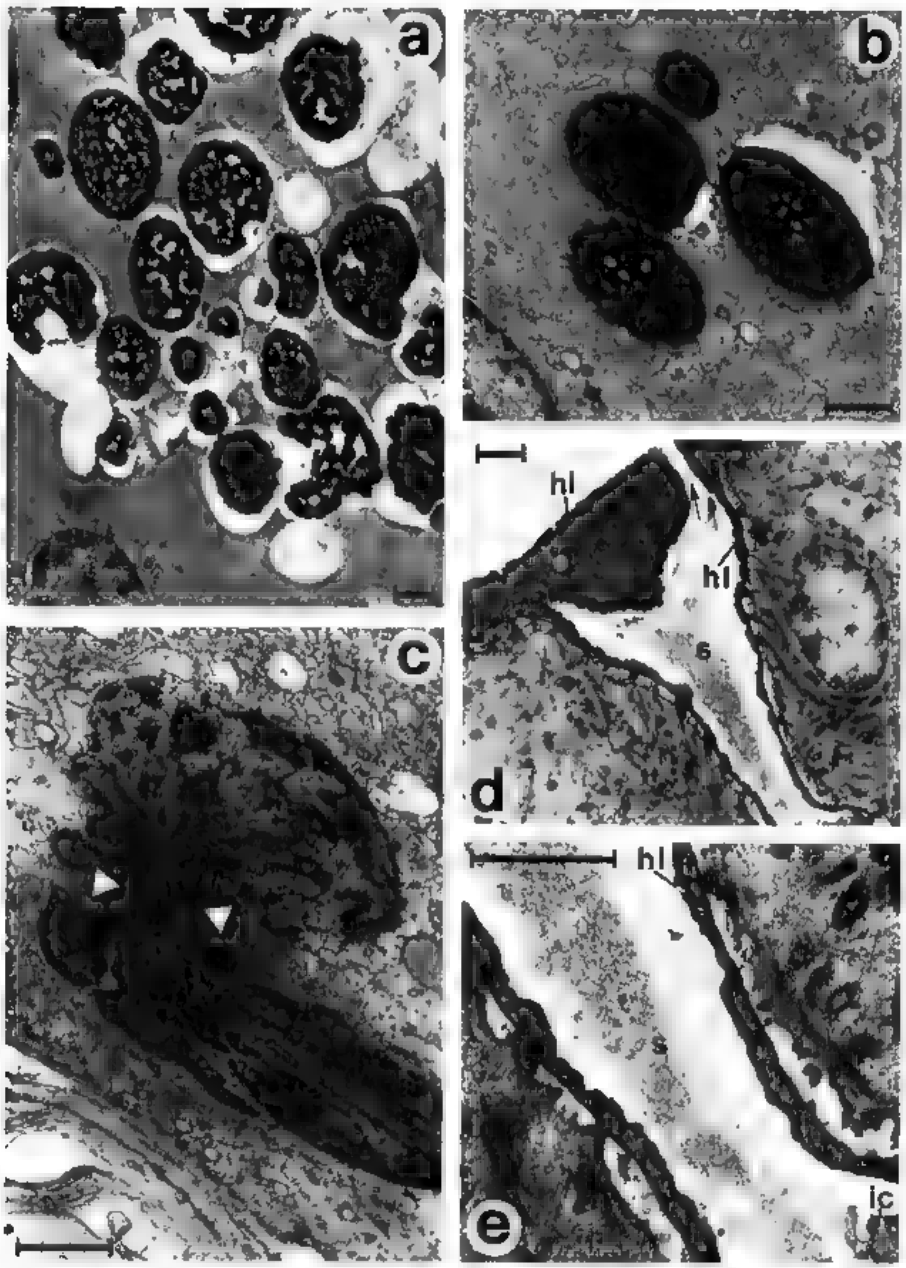

Fig. 6 – Serous glands in juveniles. Bars: 1µm.

a-b. – *Rana iberica* (a) and *Rana esculenta* complex (b): the serous product consists of granules with spongious substructure and high electrondensity; the secretory organules are restricted to the syncytium periphery.

c. – *Rana esculenta* complex in contracted myoepithelial cells the nuclei bulge toward the secretory syncytium, arrowheads indicate a dense ring of myofilaments around the nucleus which is shaped like an hourglass.

d. *Rana esculenta* complex: gland duct reaching the body surface, lined with horny layer (hl) and keratinocytes. The serous secretory product (s) consists of a structureless, moderately electrondense material; coupled arrows indicate duct outlet.

e. Enlargement of the previous micrograph, showing a detail of the duct; only cytoplasmic projections of intercalary tract cells (ic) are interposed between duct and neck lumina. s serous secretory product, hl: horny layer.

layers, bounded by a horny lining, continuous with the external *stratum corneum* of the body surface (fig. 6d). The duct lumen contains a rather opaque, structureless material, devoid of any membranous body (fig. 6e). The lack of membrane bounded serous material within the lumen is consistent with merocrine secretory release.

DISCUSSION AND CONCLUSIONS

Several ultrastructural and pharmacological investigations have depicted the discharge mechanism in serous cutaneous glands in anurans. The interstices between the secretory unit and myoepithelium hold axonal endings (BÖCK & LERTPRAPAI, 1972; WHITEAR, 1974) which form synaptic junctions with the myocytes and contain dense-cored vesicles (150 nm in diameter), adrenergic in appearance (DOCKRAY & HOPKINS, 1975; SJÖBERG & FLOCK, 1976; DELFINO, 1979, 1991; BARBERIO et al., 1987). This sympathetic innervation has been confirmed by nor-epinephrine stimulation which allowed pharmacological characterization of receptors on myoepithelial cell membranes (BENSON & HADLEY, 1969; HOLMES et al., 1977; HOLMES & BALLS, 1978; DELFINO et al., 1982). TEM observations disclosed typical ultrastructural features in still contracted mec: myocytes show alternating thickenings among myofilaments, whereas their nuclei are displaced towards the secretory syncytium; the plasmalemma facing the stroma displays several folds (DELFINO, 1980, 1991; DELFINO et al., 1987, 1990). In the Italian green frog specimens, we observed both myofilament thickenings and nuclear displacements, although the patterns were not so dramatic as in experimental specimens. The serous product is stored within the syncytium cytoplasm, so that the intense compression exerted by the myoepithelial sheath, triggered by pharmacological stimulation, causes bulk discharge of the secretory product, syncytium cytoplasm and nuclei (DELFINO, 1980). When the secretory product consists of dense particles, they can be collected in saline and were found to still possess their limiting membranes (DOCKRAY & HOPKINS, 1975, in *Xenopus laevis*). This excludes that granule release occurs through exocytosis, which involves insertion of the membrane encompassing the secretory particle into the plasmalemma. Under pharmacological stimulation, the activity of serous cutaneous glands of anurans may be regarded as *holocrine* in nature, since it involves emission of fragments of the secretory units. This bulk emission mechanism, which requires phasic contractions achieved through neuromuscular machinery, is well consistent with the defensive role ascribed to the serous cutaneous glands of anuran skin.

However, recent trends credit these secretory units, at least in *Rana* species, to perform a regulatory role in the water balance of the skin. MILLS & PRUM (1984) assign this function to specialized cells of the mucous and *seromucous* glands (the latter represent a novel type found by these authors in *Rana catesbeiana*, *Rana pipiens* and *Rana temporaria*), whereas in the *Rana esculenta* complex BARNI el al (1987) stressed the role of the secretion (*venom*) of large serous glands in controlling water balance. This is suggested by changes both in granule morphology (vacuolization) and chemical composition, fitting the annual cycle of the frog. The cycle includes two periods (activity, hibernation) with alternating changes in skin permeability. The above authors detected a

lower protein content during the winter phase of the annual cycle, whereas the amounts of 5-HT – involved in controlling ionic transport and water exchange – increased considerably.

It appears obvious that secretory mechanisms, based on holocriny, do not agree with the above findings, which witness change in granule morphology (possibly due to mobilisation of some component substances), rather than gland depletion. The enteramine (5-HT) is synthesized in the hyaloplasm and later accumulates in the granules (DELFINO, 1977) by means of an active, counter gradient mechanism (DAWSON, 1970, on entero-chromaffine cells). Conversely, biologically active proteins and 5-HT may be transferred from their storage sites (granules) into the hyaloplasm, and then towards the dermal environment with the contribution of the myoepithelial cells which may perform active transport through exo-endocytotic activity (BANI & DELFINO, 1990).

In late larval specimens and juveniles of *Rana esculenta* complex and *Rana iberica* we observed a third way of secretory release, differing both from bulk depletion and selective mobilisation of compounds stored in the granules, which involves the merocrine discharge of the serous product into the exiguous lumen, as well as moderate compression exerted by myoepithelial sheath, capable of driving the product towards the surface. In some instances, the skin poison of *Rana esculenta* complex has been observed passing through the duct, and was seen to still maintain a discrete structure (FRASCHINI, 1965). It must be stressed that the duct wall is discontinuous in structure, since it consists of funnel-like, telescopically arranged keratinocytes (DELFINO, 1976, 1991). Thus, fluidified secretory materials may spread through the intraepidermal interstice, an effect which is amplified under experimental conditions and leads to the collection of secretory product beneath the superficial horny layer (DELFINO, 1980). Following this pathway, active molecules of the serous product may exert their effect in the intraepidermal environment. Pharmacological studies have demonstrated that the contractile activity of myoepithelial cells of serous glands in anuran skin fits this functional mechanism as contraction may be modulated by adrenergic antagonist and ionic concentration (BENSON & HADLEY, 1969; HOLMES et al., 1977; HOLMES & BALLS, 1978; DELFINO et al., 1982), adjusting serous discharge to functional requirements.

This dual gland activity based on merocrine and holocrine mechanisms agrees with the phylogenetic scenery proposed by DALY et al. (1987). They postulate that anuran cutaneous toxins include ancestral regulative molecules (biogenic amines and peptides) that are widespread among living families and have been secondarily engaged in defence strategy, according to the evolution of receptor molecules in predators In our opinion the exocytotic way of release is consistent with the use of cutaneous secretions in skin homeostasis, whereas bulk discharge fits an antipredatory role.

RESUMEN

Las glándulas cutáneas serosas de ejemplares larvales y juveniles de *Rana esculenta* complex y *Rana iberica* pueden expulsar su secreción por medio de un proceso merocrino.

Tal mecanismo de secreción contrasta con la función defensiva, antipredatoria, del veneno de los anuros, basado sobre un proceso holocrino; sin embargo, el mecanismo merocrino es coherente con la ancestral función reguladora atribuida a algunos componentes de esta secreción cutánea

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